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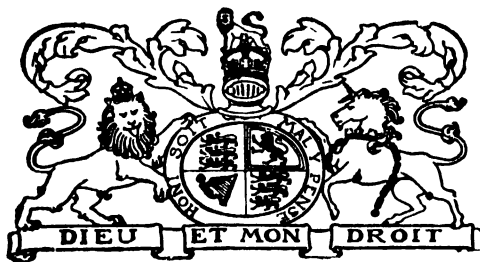
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A STUDY OF THE EPIDEMIOLOGY OF MALARIA IN A PUNJAB DISTRICT.

BY

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[17th September, 1936.]

IN 1929 Macdonald and Majid (1931) began a survey of the malarial conditions in the Karnal district of the Punjab. They published the results to the end of 1930. From 1931 the survey has been continued by A. Majid. In the present paper the results from 1929 to April 1936 are analysed by E. P. Hicks.

Karnal is in the Punjab plain, between Delhi and Ambala. It lies between those northern and south-eastern areas of the province in which fulminant epidemics have chiefly occurred (Christophers, 1911). The Jumna river runs about eight miles east of Karnal, the Western Jumna Canal runs from north to south through the district, and the bed of the old Western Jumna Canal, now disused, lies between the two. A full description of the terrain has been given by Macdonald and Majid (1931).

The villages included in the survey are Darar, Ghogripur, Indri, Kunjpura, Shahpur and Taraori. An additional mosquito-catching station has been maintained at Saidpura. At Indri, where the old Western Jumna Canal diverges from the modern canal, there is an overflow, known as the Budha Khara Escape. There are always pools of water near this village, even in the dry season. Ghogripur and Saidpura lie near the modern canal. Kunjpura is situated in low-lying ground, with high subsoil water. Shahpur and Taraori are near distributaries of the canal, and are surrounded by pools of water in the monsoon. There is also a large tank at Taraori. Darar is the least liable to flooding, as the ground falls away to the east of the village. It is the least

malarious of the villages. There are dispensaries at Indri, Kunjpura and Taraori, which distribute quinine.

The monsoon usually begins in July, and the first considerable increase of malaria is usually in September.

The villages were visited at suitable intervals in 1929, 1930 and 1931. From May 1932 to April 1936 the survey has been almost continuous. Each village is now visited once a month. The children are examined for enlargement of the spleen and the presence of parasites in the blood.

The enlargement is measured by the method of Christophers and Khazan Chand (1924). The size of the spleen is recorded as the distance in centimetres from the apex of the spleen to the umbilicus. Thus large spleens have small measurements.

Thick and thin blood films are prepared for all children by the method of Sinton (1924). Several workers have rejected this method, on the ground that it is too complicated for use in the field. It is in reality very simple. A person accustomed to the capillary pipette can use it without practice; an unskilled person can learn the method in an hour or so. Even when the films are hastily prepared, the counts are quite accurate enough for the purposes of a malaria survey, and with a little care the method gives a high degree of accuracy. Very little apparatus is required, and the time consumed is negligible. A practised person can deal with sixty children in an hour, measuring the spleens by Christophers' method and preparing blood films by Sinton's method.

It is generally believed that the epidemiology of malaria is largely governed by the balance of immunity and infection. In the case of endemic malaria, immunity and infection are more or less constant, so that severe outbreaks are unusual. In areas subject to epidemics of malaria, like the Punjab, both infection and immunity vary widely, and a severe epidemic occurs when the dose of infection is high and immunity is low. In this paper an attempt has been made to measure the factors involved in infection and immunity in a district subject to epidemics of malaria.

INCIDENCE OF MALARIA.

The history of malaria during this survey is shown in Chart 1 and Appendix I. There were considerable autumn rises in the spleen and parasite rates in 1933 and 1935, with smaller rises in the other years.

HISTORY OF BENIGN TERTIAN, MALIGNANT TERTIAN AND QUARTAN MALARIA.

Quartan parasites were found 39 times in 17,940 blood films.

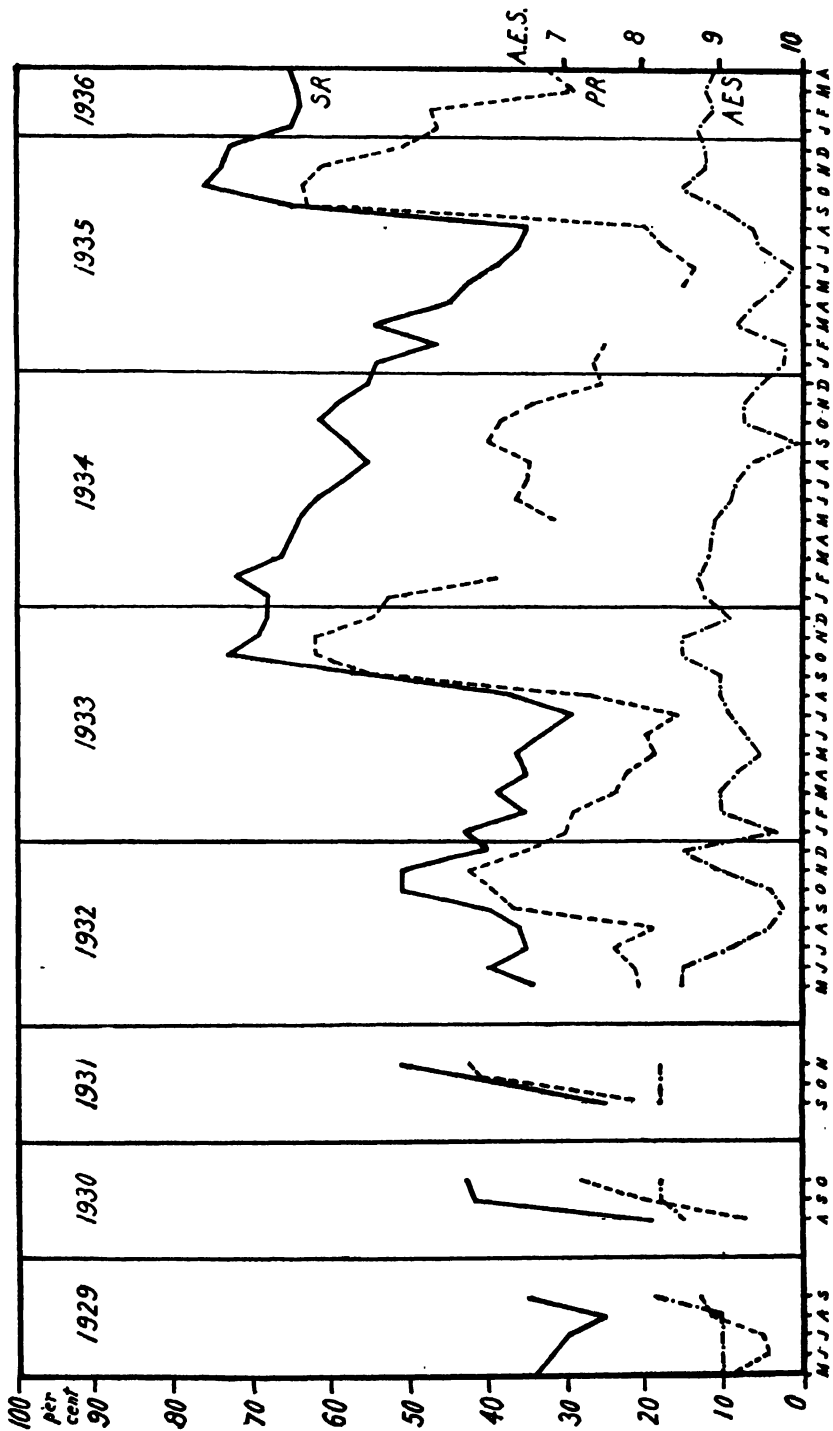
Chart 2 gives the parasite rates for benign and malignant tertian malaria. It shows :—

1. Peak of malignant tertian in November.
2. Gradual decline of malignant tertian till the following September.
3. Peak of benign tertian before that of malignant tertian.
4. A rise of benign tertian between April and June of the following year.

The malaria cases which occur from January to July cannot be due to fresh infections. Later in this paper we give reasons for believing that there is no appreciable transmission between January and June or July, and Gill (1920) has shown that in the Punjab there is no correlation of malaria with the rainfall

CHART 1.

Monthly spleen rate (SR), parasite rate (PR) and average enlarged spleen (AES) for all villages combined.

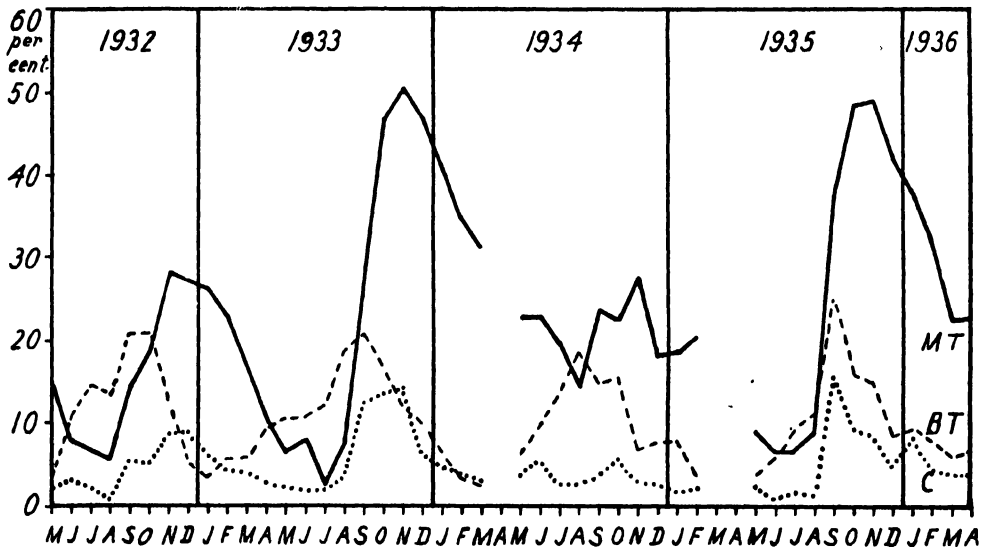


between January and June. Moreover it is difficult to understand how there can be transmission of malignant tertian in the early months of the year without the simultaneous transmission of benign tertian; and the reverse holds for the rise of benign tertian between April and June. This contrast in the incidence of the two species was recorded by Covell and Baily (1935) in Sind. It has been observed by James, Nicol and Shute (1936), working with induced malaria, and by Schüffner, Korteweg and Swellengrebel (1929) in Holland, that a person lightly infected with benign tertian often does not suffer from his primary attack till seven to ten months later.

The course of events seems to be as follows. There was an epidemic of benign tertian malaria before the beginning of the monsoon and before the

CHART 2.

Monthly parasite rates for malignant tertian asexual forms (MT), crescents (C) and benign tertian, both forms (BT). All villages combined.



period of mosquito transmission. The cases may be relapses in children who had an attack in the previous malaria season, or they may represent the first appearance of the disease in children who were infected in the previous autumn, without having a frank attack at that time. In either case, the epidemic presumably gave rise to a population of gametocyte carriers, who were ready to infect the mosquitoes as soon as transmission could begin. There was therefore a further rise in the benign tertian rate quite early in the malaria season. By the end of September, most of the population had been infected with benign tertian, and those who were not suffering from attacks were carrying latent parasites. These were refractory to further inoculation; the parasite rate declined, and did not rise again until relapses began about May or June of the following year.

The intensity of the epidemic of relapses must depend on the degree of infection in the previous autumn.

The course of events in malignant tertian was quite different. In the months immediately preceding the malaria season there was no epidemic of relapses. On the contrary, no increase in this type of malaria can be detected until several weeks after the first increase of *A. culicifacies* (*vide* Charts 7 and 8). In August, when transmission usually began, there were few gametocyte carriers. Until their number had been worked up in the early weeks of transmission no epidemic was possible. Thus the rise in the parasite rate did not begin until September, reaching its peak in November.

MEASUREMENT OF INCIDENCE OF MALARIA.

(1) FROM SPLEEN RATE.

The increase in malaria from the off-season to the malaria season is sometimes measured by the percentage increase in the spleen rate. But the resulting figure depends largely on the height of the original spleen rate. Thus a rate of 80, which rises to 100, gives a percentage increase of 25. Such a rise is due to a high degree of infection. But a rate of 20, rising to 30, gives an increase of 50 per cent, although the incidence is slight. This method, therefore, does not give an accurate estimate of the incidence of malaria.

Most of those who had enlarged spleens just before the malaria season will still have enlarged spleens in the malaria season, whether they are again infected or not. They cannot be used as an index of the malaria which has been newly acquired in the malaria season. But if we take those who had no enlargement in the off-season, and observe the number of this class which develops an enlargement in the malaria season, we can gauge the incidence of newly acquired malaria. In the imaginary instance above, 80 per cent had enlarged spleens, 20 per cent had no palpable enlargement. The spleen rate rose from 80 to 100, an increase of 20 in the malaria season. Therefore, among twenty who previously showed no enlargement of the spleen, twenty developed an enlargement in the malaria season, or 100 per cent. Since we can assume that mosquitoes distribute infections impartially among those with and those without splenic enlargement, the incidence is 100 per cent throughout the population. In the case in which the spleen rate rose from 20 to 30 per cent, the incidence is 12·5 per cent.

This method makes no allowance for the decline of the spleen rate which would have continued in the absence of fresh infection. The rate of decline in the off-seasons of 1933, 1934 and 1935 has been approximately constant in each year (*vide* Chart 1). This rate can be calculated (Table I).

These rates are of course not of general application. They apply only to the population and period under review. But it may be assumed that they would have continued for two or three months in the absence of fresh infection. The calculation of the rate of decline for each year has therefore been continued to the mid-point of the malaria season, in order to estimate what the value of the spleen rate would have been in the absence of fresh infection. From this estimate, and from the mean value of the spleen rate actually observed in the period September to December, it is possible to calculate an index of incidence by the method set out above. Table II gives the indices obtained. The index for 1932 may be too low, as the figures for the spleen rates in May to August give no evidence of a decline. The values of the index for the individual

Study of Malaria in a Punjab District.

TABLE I.
Rate of decline of spleen rate.

	1933		1934		1935	
	Observed.	Calculated.	Observed.	Calculated.	Observed.	Calculated.
Monthly rate of decline (per cent).	5.9		3.2		5.3	
Spleen rate for January ..	42.7	42.4	68.2	71.0	54.1	52.7
" February ..	35.1	39.9	71.5	68.7	45.9	49.9
" March ..	38.5	37.5	66.0	66.5	54.0	47.3
" April ..	35.0	35.3	..	64.4	45.3	44.8
" May ..	36.0	33.2	63.8	62.3	42.5	42.4
" June ..	33.0	31.3	61.9	60.4	38.6	40.1
" July ..	28.6	29.4	57.9	58.4	35.6	38.0
" August	54.6	56.6	35.2	36.0
Sum	248.9	249.0	443.9	443.9 + 64.4	351.2	351.2

TABLE II.
Indices of incidence of malaria, calculated from the spleen rate.

	1932	1933	1934	1935
Darar ..	7	46	0	57
Ghogripur ..	12	40	14	61
Indri ..	6	86	21	73
Kunjpura ..	37	57	0	64
Shahpur ..	28	44	55	82
Taraori ..	32	64	0	42
Six villages ..	14.0	55.8	12.3	58.7
<i>r</i> ..	15	81	13	88

Calculation of index of incidence. The method is more easily explained by an example. The following is the calculation for 1933 for the six villages combined:—

Calculated spleen rate for July (from Table I) 29.4 per cent.

Calculated rate for mid-point of the period September to December 23.8 "

Percentage without enlarged spleens, 100—23.8 or .. 76.2 "

Mean observed rate, September to December 66.3 "

Increase of rate, 66.3—23.8 or .. 42.5

42.5 spleens appearing in 76.2 who previously had no enlargement gives an index of .. 55.8

villages contain considerable statistical errors, as the monthly spleen rates are based on the examination of only forty to one hundred children in each case.

According to Christophers (1915), 'the intensity of malarial infection in a community is the number of *splens* distributed in the period r , which is r . The direct measure of malaria is therefore r '. The values of r for each value of the index of incidence are also given in Table II.

(2) FROM DEATH RATE.

A second measurement of incidence is given by the epidemic figures, calculated from the death rates. They are given in Table III, in which the indices of incidence are repeated for comparison.

TABLE III.

Epidemic figures and indices of incidence for all villages combined.

	1929	1930	1931	1932	1933	1934	1935
Epidemic figure ..	0.81	1.19	1.34	1.72	2.21	0.95	1.99
Index of incidence	14.0	55.8	12.3	58.7

Calculation of epidemic figures. These are based on records of deaths from all causes in children up to ten years of age in the six villages of the survey. The epidemic figure is found by dividing the off-season death rate into the malaria season death rate. The off-season death rate is the mean monthly number of deaths in the months of January to August for the years 1929 to 1935 combined. The malaria season death rate is the mean monthly number of deaths in the months of September to December. This was calculated separately for each year.

The index of incidence and the epidemic figure give no measurement of infection or immunity. They are intended only as an estimate of incidence, which is held to be the resultant of infection and immunity.

From the above observations it is clear that during the period under review there has been no major epidemic of malaria; that the epidemics of 1933 and 1935 were above the average of the period; and that of 1934 was below the average. The failure of the 1934 epidemic might be due to a failure of transmission, or to increased immunity after the 1933 epidemic, or to both. The probability is that transmission was below the average. An average or high degree of transmission, encountering an immunity which had been raised by the 1933 epidemic, would be expected to reinforce that immunity and prevent the epidemic of 1935. An average or high degree of transmission, meeting an immunity which had not been raised by the 1933 epidemic, would be expected to produce a greater incidence of malaria than was actually observed in 1934. We therefore believe that there was failure of transmission. This hypothesis is supported by other evidence which is given later.

IMMUNITY.

In this section the statistics which are usually calculated from blood and spleen records are examined, in order to find those which are most useful in measuring immunity, and in tracing its changes from year to year.

Increased immunity means increased tolerance of a given dose of infection. Since we do not know that each village had the same dose of infection, we

cannot detect variation in immunity by comparing one village with another. But we can assume that in each village the same dose of infection was distributed to children of all ages. This assumption can be extended to the combined population of the six villages if the number of children examined in each age group is the same in all villages. Unfortunately the latter is not true. There are variations from village to village, but they are not large enough to offset the advantage of working with larger numbers. For this reason, most of the following tables and charts are based on the figures for all villages combined. Under these circumstances the value to be attached to any phenomenon is increased when it is found to be repeated over several years.

As usually happens in backward communities, the recorded age of a child is a guess based on its size. But different observers do not always assign the same age to a given size. It would perhaps be an advantage in future to group the children according to their size, which may conveniently be measured by the nipple-umbilicus distance. This would at least substitute a measurement for a guess.

MORTALITY BY AGE GROUPS.

Table IV shows the number of deaths from all causes. The non-malaria season deaths are those recorded for the months of January to August, beginning

TABLE IV.

Deaths from all causes, arranged by age groups.

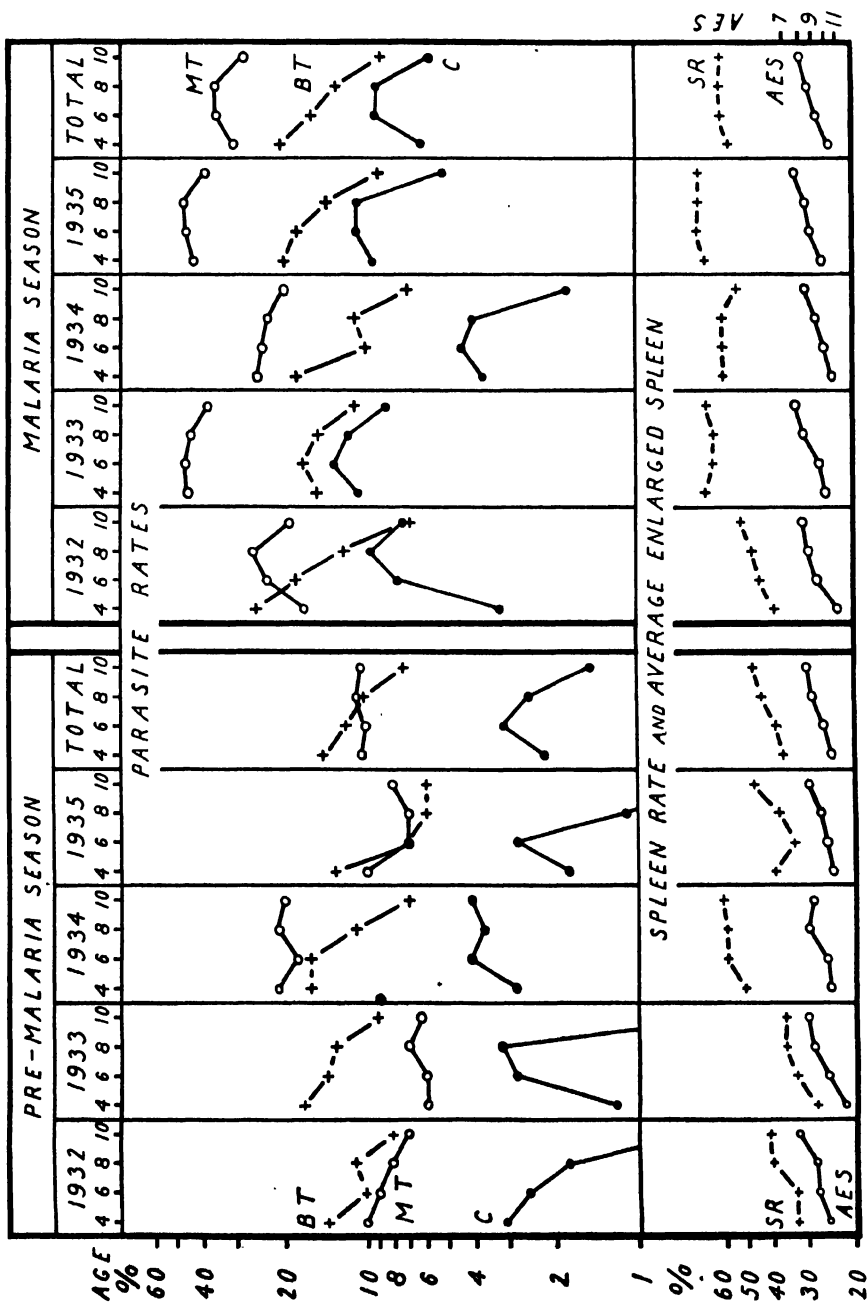
		Age in years.						
		0-2	-4	-6	-8	-10		
Non-malaria season.	Observed deaths 56 months.	614	78	31	20	12		
	Expected deaths 28 months.	307	39	15.5	10	6		
Malaria season.	Observed deaths 28 months.	413	78	36	17	8		
	Excess deaths ..	106	39	20.5	7	2	Total 174.5	$\chi^2=112.17$
	Expected excess	47.89	40.27	33.90	28.50	23.94	174.5	$P=\text{less than } 0.01$

Significance of mortality by age groups. The age composition of the population is unfortunately uncertain, since the parents do not know the ages of their children exactly. The Census figures for the Karnal district do not inspire confidence. The Superintendent of Census Operations has corrected and smoothed the age composition of the Punjab population, but writes, 'The results.....have a spurious appearance of validity, which is, in reality, quite illusive'. We have adopted a hypothetical distribution, which is weighted at the younger end (Table V).

with January 1929 and ending with August 1935, that is, fifty-six months. These numbers have been reduced to the numbers expected in twenty-eight months. The malaria season deaths are those recorded in the months of

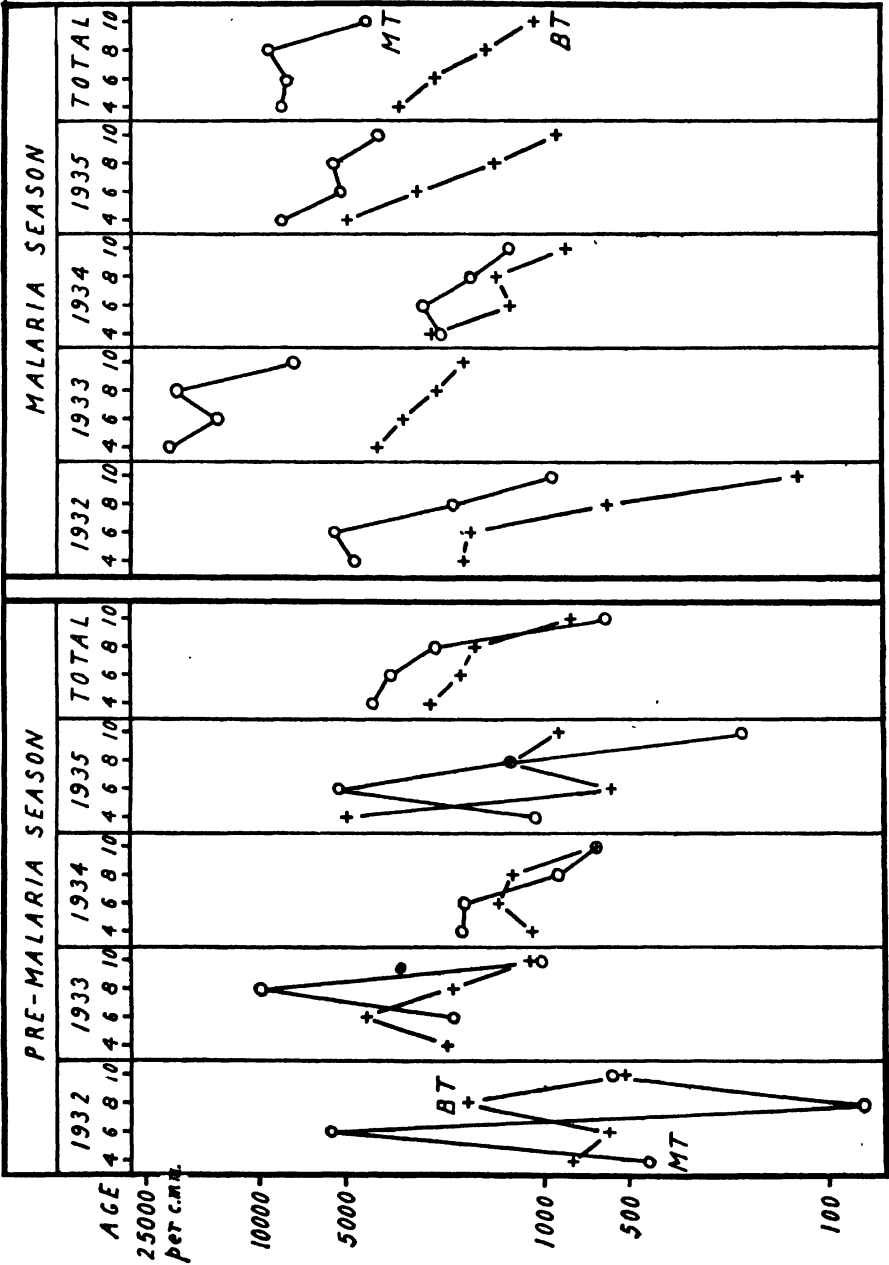
CHART 3.

Parasite rates, spleen rates (SR) and average enlarged spleen (AES) by age groups. BT, benign tertian; MT, malignant tertian asexual forms; C, crescents. The scale shows the upper limit of each age group.



September to December for the same seven years. The table shows that in the malaria season there is an excess of deaths over the number recorded for the off-season, and that this excess of deaths is concentrated at the younger end of

CHART 4.
Average parasite counts by age groups.



the scale. There is therefore in these villages the same increase of immunity with age as has been observed elsewhere. The older children did not often die when exposed to the degree of infection prevailing during this period. But it may be noticed that the immunity was slowly and gradually acquired.

TABLE V.
Age composition of population.

Age in years.	NUMBER PER 100,000 MALES IN THE PUNJAB.	
	Corrected and smoothed.	Hypothetical distribution.
Under 2	7,186	10,000
Over 2 and under 4 ..	6,252	8,410
" 4 " 6 ..	5,728	7,080
" 6 " 8 ..	5,338	5,950
" 8 " 10 ..	5,024	5,000

The excess number of deaths, 174.5, has been arranged according to this hypothetical distribution, to give the number expected in each age group if there had been no increase of immunity with age ('Expected excess'). The difference between the expected and observed distribution of deaths is undoubtedly significant.

PARASITE FINDINGS BY AGE GROUPS.

Parasite rates by age groups are shown in Chart 3 and Appendices III, IV and V. The chart is drawn to a logarithmic scale, so that equal rates of increase or decrease may be represented by equal gradients. The figures are analysed more exactly in Table VI. Chart 4 and Appendices VIII and IX show the average parasite counts by age groups. The average parasite count was found by dividing the sum of all parasite counts by the number of children who showed parasites in the blood. Only children over two years of age are included in the charts, as the numbers in the youngest group are small. The

TABLE VI.
*Comparison of number of parasite carriers in two age groups.
Benign tertian, sexual and asexual forms.*

Age in years.	PRE-MALARIA SEASON.			MALARIA SEASON.		
	Number with parasites.	Number without parasites.	Parasite rate.	Number with parasites.	Number without parasites.	Parasite rate.
Over 2 to 8	504	3,834	11.6	689	3,716	15.6
9 and 10 ..	97	1,227	7.3	96	1,000	8.8
χ^2 ..	19.67		..	34.57		..
P ..	less than 0.01 significant		..	less than 0.01 significant		..

TABLE VI—concl'd.
Malignant tertian asexual forms.

Age in years.	PRE-MALARIA SEASON.			MALARIA SEASON.		
	Number with parasites.	Number without parasites.	Parasite rate.	Number with parasites.	Number without parasites.	Parasite rate.
Over 2 to 8	455	3,883	10.5	1,533	2,872	34.8
9 and 10 ..	139	1,185	10.5	310	786	28.3
χ^2	17.48		..
P	less than 0.01		..
				significant		

malaria season is September to December, the pre-malaria season from May to August.

For benign tertian there is a decline with advancing age in the frequency of parasite carriers who are discovered by the ordinary methods of examination. Table VI shows that this decline is significant, and Chart 3 shows that it is consistent and considerable in degree. The same decline is seen in the average parasite count, though less consistently. This does not mean that fewer of the older children have infections, since there is no corresponding decline in the spleen rate. But it shows that the older children relapse less frequently in the pre-malaria season, and react less obviously to infection in the malaria season.

In malignant tertian the phenomenon is more obscure. In the pre-malaria season there is no decline with age in the frequency of parasite carriers. In the malaria season there is a decline, but only in the oldest group. Table VI shows that the decline is significant, but Chart 3 shows that it is much less than in the case of benign tertian.

We interpret this as showing that immunity has been acquired towards both types, but that immunity has been acquired more slowly to malignant tertian than to benign tertian.

The evidence from the mortality tables and from the parasite findings therefore shows that the older children have more immunity to malaria than the younger.

SPLEEN FINDINGS BY AGE GROUPS.

Spleen findings are given in Chart 3 and Appendices VI and VII.

In the pre-malaria season the spleen rate rises in the older children, whom we have shown to be more immune. Here there is a relation between a high spleen rate and a high degree of immunity. But in the malaria season the level of the spleen rate is higher, and is roughly the same at all ages in every year but one. In this season it appears to be an indication of the number of infections, and gives no evidence of the varying degrees of immunity which have been shown to exist.

The size of the average enlarged spleen shows a steady and persistent increase with age. Its variation from season to season, and from year to year, is much less than that of the other indices. It is therefore less affected by the differing doses of infection which are inflicted in the malaria season. It will be examined in greater detail, as we propose to use it as a measure of immunity.

AVERAGE ENLARGED SPLEEN.

It is known that the size of the average enlarged spleen varies with the spleen rate. Table VII shows that this was so in our survey. In this table, the average enlarged spleen in each village for each month is correlated with the corresponding spleen rate. The correlation is inverse, because large spleens have small measurements.

TABLE VII.

Correlation between the average enlarged spleen (AES) in each village for each month with the corresponding spleen rate (SR). Spleen rates below 10 per cent are excluded.

AES.	Spleen rate.								
	10 +	20 +	30 +	40 +	50 +	60 +	70 +	80 +	90 +
6.0 +	1	1	..
6.5 +	1	1	3
7.0 +	1	1	2	..	5	2	2
7.5 +	2	3	2	9	4	2	4
8.0 +	1	..	4	4	6	7	8	7	4
8.5 +	..	4	3	5	6	11	11	5	..
9.0 +	3	2	4	8	13	13	6	3	..
9.5 +	1	9	5	7	12	11	4	2	..
10.0 +	10	7	10	10	11	7	1
10.5 +	2	5	6	..	5	1	..	1	..
11.0 +	9	2	3	2	..	1
11.5 +	8	..	1	1

Coefficient of correlation = -0.565 ± 0.025 .

Regression, AES = $10.864 - 0.029$ SR.

SR = $153.27 - 10.82$ AES.

It appears at first sight that the average enlarged spleen is no more than a function of the spleen rate. But when the data for each individual village are plotted against the regression line of the whole community (Chart 5), it will be seen that another factor is involved. For one thing, the correlation is of a much lower order (Table VIII and Appendix X).

TABLE VIII.

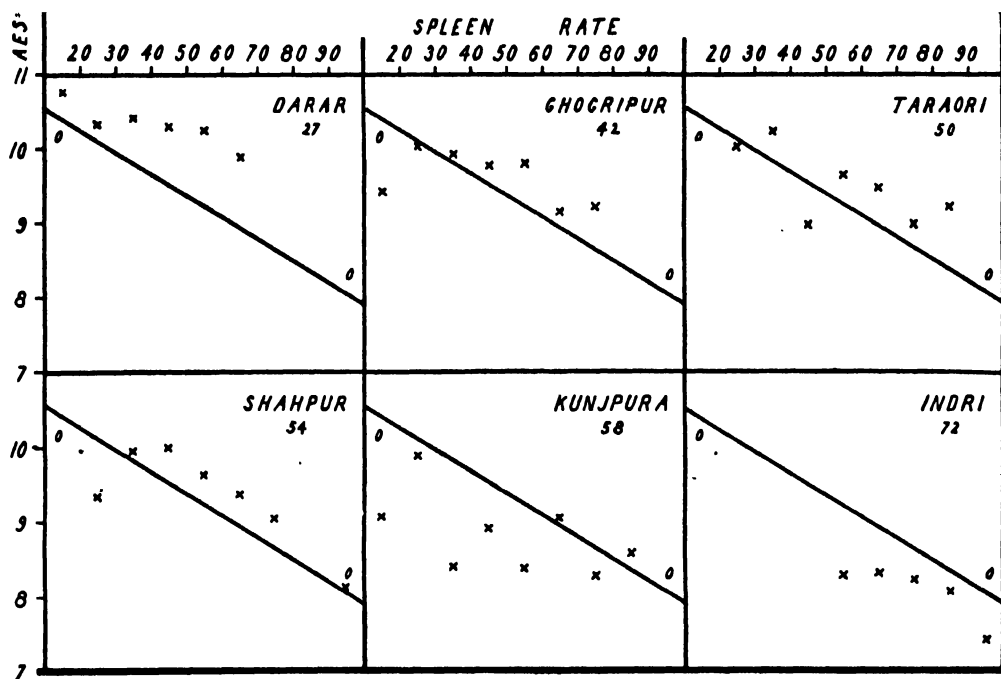
Correlation between average enlarged spleen and spleen rate.

	<i>n.</i>	Correlation coefficient.	<i>t.</i>	<i>P</i> (approx.).
All villages	338	-0.565	-12.51	less than 0.01
Darar	55	-0.306	-2.34	0.025
Ghogripur	54	-0.136	-0.99	0.32
Indri	65	-0.297	-2.47	0.015
Kunjpura	54	-0.243	-1.80	0.075
Shahpur	55	-0.450	-3.67	less than 0.01
Taraori	55	-0.293	-2.23	0.03

Again, in those villages, such as Indri, which have a constant high spleen rate, the average enlarged spleen is larger than would have been expected from the regression line of the whole community. But in Darar, which usually has a low spleen rate, the average enlarged spleen is smaller than expectation, and remains smaller even when the spleen rate rises. In fact, the average enlarged spleen does not vary so closely with the spleen rate as might have been inferred

CHART 5.

Mean value of average enlarged spleen for each value of the spleen rate.
 0—0, regression line of average enlarged spleen on spleen rate for whole community (from Table VII). The figure beneath the name of the village is the mean spleen rate.



from the correlation table. Each village has a tendency to adhere to its own size of enlarged spleen, which is related to its mean spleen rate. The village which has a constant high spleen rate, and presumably a high degree of immunity, tends to contribute most of the large spleens seen in Table VII, while the village which usually has a low spleen rate, and presumably less immunity, furnishes most of the small spleens.

This evidence confirms the hypothesis of Christophers (1929). 'It looks, however, as though there might be, besides the normal spleen, (a) two kinds of enlarged spleen, (b) the spleen of acute malaria, and (c) the spleen of immune malaria, and that each of these spleens is biometrically distinct, i.e., with its own mean size and frequency. (b) can be envisaged as ranged in frequency towards the costal margin end of our base line with its mean about say 10 cm.;

and (c) with a range more towards the umbilicus end of the line with its mean say at 6 cm.

'The size of the spleen, if this view be correct, will still *usually* appear as a function of the spleen rate because the more intense the malaria (provided it is sufficiently permanent) the more children will have spleens and the more the large immunity spleens will come in. But if the malaria is not static there may be a high spleen rate with a low average spleen'.

If the average enlarged spleen is a measure of immunity, then children with large spleens should have lower parasite rates and counts. Table IX gives the parasite rates and counts for benign tertian. This type has been chosen as giving better evidence of immunity than malignant tertian.

TABLE IX.

Relation of benign tertian parasite rate and average parasite count to size of spleen. The average counts recorded by Christophers (1924) in the hyper-endemic Singhbhum area are given for comparison.

	Not palpable.	APEX-UMBILICUS MEASUREMENT.							Beyond umbilicus.
		14 and over.	13-12	11-10	9-8	7-6	5-4	3-0	
<i>Number of children examined.</i>	2,241	142	580	1,006	789	522	232	105	51
<i>Parasite rate ..</i>	10.1	16.9	22.4	18.2	14.9	17.2	12.9	9.5	5.9
<i>Average parasite count.</i>	1,380	2,840	3,150	2,320	2,060	1,760	3,430	760	430
<i>Average count, Singhbhum.</i>	716	933	1,000	4,800	7,900	510	380

The parasite rate is highest with spleens of 13-12 cm., and tends to decrease with larger spleens. Perhaps the same tendency may be detected in the average parasite counts. Table IX also gives for comparison the parasite counts (all species) obtained by Christophers (1924) in the Singhbhum district, Orissa. Here the peak at 9-8 cm. is much more clearly distinguished. The Singhbhum district was one of hyperendemic malaria, with a spleen rate of about 70 per cent. Infection was more constant and more intense than in the Karnal district. Probably the immunity was more quickly acquired and rose to a higher degree. The Karnal children show the same phenomenon as the Singhbhum children, though less obviously. Thus the larger spleens are associated with higher immunity.

MEASUREMENT OF IMMUNITY.

Mortality is the resultant of infection and immunity. It gives no index of immunity, unless the dose of infection is constant.

The parasite rate and average parasite count vary with immunity, but even more with infection. They are therefore of little use for tracing changes of immunity from year to year.

The spleen rate is an indication of the number of infections received. A constant spleen rate is the result of a constant level of infection, and so

presumably of a constant level of immunity. This is the condition in an area of endemic malaria. But in an area of epidemic malaria, such as the greater part of the Karnal district, the spleen rate fluctuates widely. In the malaria season its level was an index of the degree of infection, and it showed no evidence of the differences in immunity which were found between the various age groups. In the pre-malaria season the indication of immunity is clearer. But it is difficult to separate it from the effects of the residue of infection from the previous malaria season. A fluctuating spleen rate is therefore of little help in tracing changes of immunity from year to year. While a low rate may show absence both of immunity and of recent infection, a high rate may be due either to recent infection in a non-immune population, or to a constant high level of infection in an immune population.

The average enlarged spleen shows a definite gradation which corresponds to the gradation of immunity revealed by the mortality table, the parasite rate and the average parasite count. The change from the pre-malaria to the malaria season is slight. Since it is the measurement of immunity which is least affected by temporary changes in the dose of infection, it will be used for tracing changes in immunity throughout the period of this survey.

Appendix I gives the value of the average enlarged spleen for each village and each month. The last three lines show the means, standard deviations and coefficients of variation.

The changes from month to month are considerable but irregular. They are no more than might be due to errors of random sampling. Chart 1, however, shows some evidence of seasonal variation. In this chart the curve for the average enlarged spleen is drawn to a large scale, and the fluctuations are therefore exaggerated. But the curve does show a rise in the malaria season. The rise following the major malaria seasons of 1933 and 1935 is definite and significant, but that following the malaria seasons of 1932 and 1934 is slight and not significant (Table X). In some villages there was a decrease after 1932 and 1934.

TABLE X.

Rise of average enlarged spleen after the malaria season.

	1932 and 1934.	1933 and 1935.
Mean increase in cm.	0.30	0.98
<i>t</i>	1.417	6.732
<i>P</i>	between 0.1 and 0.2	less than 0.1
	not significant	significant

Significance of change in average enlarged spleen. (a) The mean value of the average enlarged spleen for May to August was found for each village. (b) Similarly the mean value was found for the months of January to April following. (c) The differences of (a) and (b) for each village were arrayed in one table for the two years 1932 and 1934, and in a second table for 1933 and 1935. (d) The mean of each table was determined, and its significance was tested by calculating *t* and *P* (Fisher, 1934).

The malaria seasons of 1933 and 1934 therefore probably caused an increase of immunity. But Chart 1 shows that it was of short duration. We conclude that there has been little change in the immunity of the population during the period of our survey.

RELATION OF PARASITE RATE TO SPLEEN RATE.

The parasite rate in children with enlarged spleens (Table XI) will be useful in comparing one village with another. In each year it rises from the pre-malaria to the malaria season according to the incidence of malaria. Thus a low rate indicates the absence of recent severe malaria. This was the

TABLE XI.

Parasite rate (all species) in children with enlarged spleens. The ages shown are the upper limits of each age group.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	55	51	39	34	23	84	69	69	58	35
1933	..	56	44	35	23	..	76	78	69	58
1934	..	65	46	44	34	53	60	51	47	35
1935	73	41	26	19	20	82	73	70	70	63
Four years.	56.3	50.7	39.6	34.5	26.5	75.4	69.3	68.2	62.4	48.2

experience of Barber, Rice and Valaoras (1936) in East Macedonia. 'A small divergence between the spleen and parasite indexes is here associated with high transmission, a great divergence with low transmission.....In a single survey of school children, one may, by comparing parasite and spleen indexes, obtain a clue to the amount of transmission during the preceding year or years'. They refer to the total parasite rate in all children.

On the other hand, the parasite rate in children with enlarged spleens is lower in the more immune children. Thus a divergence between this rate and the spleen rate is also evidence of increased immunity. The rate rises with increased incidence of malaria, but falls with increased immunity.

CRESCENT RATE AND IMMUNITY.

It has been shown that the malignant tertian parasite rate (asexual) declines in the oldest and most immune group of children during the malaria season. Chart 3 shows that in this season the crescent rate declines in the same ratio. The figures are analysed in Table XII, which shows that the frequency of crescent carriers among children infected with malignant tertian is not significantly different in the oldest children.

TABLE XII.

Comparison for two age groups of the number of crescent carriers among those children who showed malignant tertian parasites in the blood (malaria season only).

Age in years.	Number with crescents.	Number without crescents.	Percentage.
Over 2 to 7	378	1,264	23
8 and 9	63	267	19
χ^2	2.449		..
P	0.1 approx. not significant		..

There is no evidence that such immunity as has been acquired by the children of the Karnal district towards malignant tertian has caused a greater reduction of sexual than of asexual forms.

The higher immunity of those who live in hyperendemic areas may perhaps have some specific action on crescents. Schüffner *et al.* (1932) calculated for different ages the ratio of crescent carriers to malignant tertian carriers in three separate surveys. Those in Mandailing (Sumatra) and in South Africa were in hyperendemic districts. The third was in Sundatar (Sumatra) during an epidemic following several years without serious malaria. Table XIII, which has been adapted from their figures, shows for two age groups the frequency of crescent carriers among those with malignant tertian.

TABLE XIII.

Comparison for two age groups of the number of crescent carriers among those children who showed malignant tertian parasites in the blood. Adapted from Schüffner *et al.* (1932).

Age in years.	SUNDATAR (EPIDEMIC).			MANDAILING (HYPERENDEMIC).			SOUTH AFRICA (HYPERENDEMIC).		
	Number with crescents.	Number without crescents.	Per cent.	Number with crescents.	Number without crescents.	Per cent.	Number with crescents.	Number without crescents.	Per cent.
2-5	47	22	68	83	153	35	42	79	35
6-9	65	28	70	30	97	24	36	162	18
χ^2	0.058		..	5.130		..	11.082		..
P	0.9 approx. not significant		..	0.02 approx. significant		..	less than 0.01 significant		..

Thus the crescent rate declined more rapidly than the malignant tertian rate in the children who lived in hyperendemic areas, but not in those who were suffering from an isolated epidemic. The decline may be due to a specific action of immunity on gametocytes, or it may be due to the scarcity of crescents in the peripheral blood when the crescent rate is low. For example, in Karnal during the months of May, June and July the average crescent count was

106 per c.mm., while the average count of asexual forms was 1,057 per c.mm. Of the two, the crescent count was much nearer the level below which many individual carriers escape detection.

INFECTION.

PREVALENCE OF ANOPHELES.

The seasonal prevalence of eight species is shown in Table XIV. All figures relating to adult mosquitoes are taken from the monthly catches in the villages and from the weekly catches at Saidpura.

TABLE XIV.

Seasonal prevalence of mosquitoes. The monthly prevalence of each species is shown as a percentage of the total catch of that species for the twelve months. Calculated from the mean monthly catch in each village. Female mosquitoes only. Period, May 1932 to April 1936.

	<i>A. culicifacies.</i>	<i>A. subpictus.</i>	<i>A. annularis.</i>	<i>A. stephensi.</i>	<i>A. fluviatilis.</i>	<i>A. pallidus.</i>	<i>A. splendens.</i>	<i>A. pulcherrimus.</i>
January ..	1.6	0.1	4.9	4.8	19.3	1.0	2.1	0
February ..	0.2	0	13.6	7.1	6.3	0	13.8	4.4
March ..	0.5	0	19.4	8.7	25.0	0	26.6	4.4
April ..	1.3	0.1	11.2	15.9	6.3	0	11.7	1.2
May ..	4.2	1.1	5.8	9.5	4.1	0	6.4	0.5
June ..	6.8	5.2	3.2	4.8	0.3	0.3	6.4	0
July ..	15.0	20.9	2.7	11.1	0.3	4.5	11.7	5.8
August ..	24.5	29.2	3.6	7.9	0.6	18.3	6.4	5.8
September ..	20.6	17.0	4.7	13.5	0	23.8	3.2	19.1
October ..	9.9	14.9	5.9	5.6	1.9	20.7	1.1	26.2
November ..	10.8	8.9	14.1	5.6	20.9	21.4	4.3	26.2
December ..	4.2	2.9	10.9	5.6	14.9	10.0	6.4	6.2

In each village the collector visited certain cattle sheds, catching the mosquitoes with the aid of a torch and test tubes. The catches were made during the morning, and an approximately equal time was spent on each occasion. The search was made in cattle sheds because the villagers will seldom allow the collector to enter their houses. By this method there is always the possibility that the mosquitoes which are caught do not represent a fair sample of the mosquitoes which bite human beings. To test this, we have compared the number of adult mosquitoes caught with the number of times the corresponding larvæ were taken (Table XV). The percentage frequency is roughly the same in each case. Therefore we believe that our catches represent a fair sample of the mosquito population.

It is usual to compare catches made in a standard time, but perhaps space would furnish a better standard than time. When mosquitoes are scarce, a man may catch all the mosquitoes in a room in less than the appointed time. But when they are swarming in large numbers, he may catch only a small proportion. If, however, the collector was instructed to catch all the mosquitoes

TABLE XV.

Relative frequency of adult and larval mosquitoes. Period, May 1932 to April 1936.

	ADULT FEMALES.			LARVÆ.	
	Total number captured.	Percentage of all mosquitoes.	Mean catch July to September.	Number of times captured.	Percentage of all captures.
<i>A. subpictus</i> ..	25,987	43.8	102	810	40.5
<i>A. annularis</i> ..	21,772	36.6	15	486	24.3
<i>A. culicifacies</i> ..	8,344	14.0	37	412	20.6
<i>A. pallidus</i> ..	1,407	2.4	4.6	37	1.9
<i>A. fluviatilis</i> ..	873 *	1.5	0.1	7	0.4
<i>A. stephensi</i> ..	483	0.8	1.4	17	0.9
<i>A. pulcherrimus</i> ..	297	0.5	0.6	5	0.3
<i>A. splendidus</i> ..	268 †	0.5	0.7	4	0.2
<i>A. hyrcanus</i> ..	42	0.1	..	112	5.6
<i>A. barbirostris</i> ..	5	102	5.1
<i>A. turkhudi</i> ..	3	1	..
<i>A. maculatus</i> ..	1	3	..
<i>A. jamesi</i> ..	1	1	..
<i>A. gigas</i> ..	0	2	..
TOTAL ..	59,483	1,999	..

* 87 per cent caught in Indri.

† 78 per cent caught in Indri.

in a room, however long the time, and if he visited the same room each week, a better estimate of variation in the mosquito population would be secured.

CARRIER SPECIES.

Table XV also shows the mean catches for the months of July, August and September. *A. subpictus*, *A. annularis* and *A. culicifacies* are numerous enough to

TABLE XVI.

A.

Dissections of mosquitoes in Karnal area, July 1931 to January 1936. A mosquito which had both oöcysts and sporozoites is entered once under oöcysts and once under sporozoites.

	Number dissected.	PERCENTAGE INFECTED	
		oöcysts.	sporozoites.
<i>A. culicifacies</i> ..	8,815	0.23	0.36
<i>A. fluviatilis</i> ..	381	0	0.26
<i>A. stephensi</i> ..	254	0	0.39
<i>A. annularis</i> ..	206	0	0
<i>A. subpictus</i> ..	159	0	0
<i>A. splendidus</i> ..	98	0	0

TABLE XVI—concd.

B.

Dissections of A. culicifacies, arranged by months.

Year.	JANUARY TO JULY.			AUGUST.			SEPTEMBER.			OCTOBER.			NOVEMBER.			DECEMBER.		
	Number			Number			Number			Number			Number			Number		
	dissected.	infected		dissected.	infected		dissected.	infected		dissected.	infected		dissected.	infected		dissected.	infected	
		gut.	glands.		gut.	glands.		gut.	glands.		gut.	glands.		gut.	glands.		gut.	glands.
1931	..	23	0	0	86	0	206	1	1	107	0	1	153	0	5	8	0	0
1932	..	7	0	0	247	4	497	7	1	206	0	1	280	0	2	78	1	0
1933	..	697	0	0	476	0	323	0	0	358	1	2	22	0	0	0
1934	..	318	0	0	411	1	439	0	3	476	0	0	370	0	0	242	0	0
1935	..	1,004	0	0	250	3	210	2	3	194	0	1	157	0	0	111	0	1
1936	..	59	0	0
Total	..	2,108	0	0	1,470	8	1,675	10	8	1,341	1	5	982	0	7	439	1	1
PER CENT.	..	0	0	..	0.54	0.75	..	0.60	0.48	..	0.07	0.37	..	0	0.71	..	0.23	0.23

carry malaria, even if lightly infected. *A. pallidus* and *A. stephensi* might do so if heavily infected.

Table XVI gives the results of dissections. It is based on mosquitoes caught in all parts of the Karnal area. *A. culicifacies* is obviously a carrier. For the other species, the number dissected is too small to give a reliable sporozoite rate. In 254 dissections, *A. stephensi* was found infected once. In view of this, and of its reputation in other parts of India, it must be considered a carrier in the Karnal district. But its numbers are so small that it can be responsible for a very little transmission. *A. subpictus* may be excluded on the results of dissections in India and the East. In 9,294 dissections recorded by Covell (1931) it was found to be infected once only. *A. fluviatilis* is scanty in the malaria season. In 1,718 dissections in India, oöcysts have been found three times, sporozoites never (Covell, *loc. cit.*). It can hardly be responsible for transmission in this district. *A. pallidus* has rarely been found naturally infected. In 1,552 dissections, three gland infections were found (Covell, *loc. cit.*). In another series of 712 dissections by Sur (Bose, 1931), oöcysts were discovered in one specimen. It is improbable that it is a natural carrier of importance to Karnal.

For these reasons we conclude that *A. culicifacies* is the only carrier of importance in the Karnal area.

TRANSMISSION SEASON.

Examination of the parasite rates and average parasite counts shows that the first increase of malignant tertian malaria occurred on the following dates :—

1932	14th September.
1933	29th August.
1934	18th September (indefinite onset).
1935	27th August.

Probably few children were infected with malignant tertian before the 1st August. The epidemic of relapses of benign tertian makes it difficult to time the transmission of this species. The date on which mosquitoes acquire the power of transmission is presumably the same for both species. But the relapses will have provided a larger population of carriers of benign tertian gametocytes, and transmission of this species may begin a little earlier.

A consideration of the numbers captured and the monthly sporozoite rates also points to August and September as the months of maximum transmission. Of 844 *A. culicifacies* dissected in the months of July, not a single mosquito was found infected. The earliest infection we have found was in a mosquito captured on the 2nd August in 1932. We therefore consider that, during this survey, the children were rarely infected before the beginning of August. But the climate of July is important, because it determines the time when mosquitoes can increase, and receive infection. Gill (1920) has shown that July is the first month in which there is definite correlation between rainfall and malaria.

After September transmission declines. Sporozoite infections are infrequent in November and December. Of the five infected mosquitoes captured in November 1931, four were from Budha Khera, a village on the banks of the Budha Khera Escape. It is possible that the Escape was flooded at the time, with the formation of pools and a local rise of humidity.

CLIMATE AND MALARIA.

The meteorological data were recorded in the Ross Field Experimental Station for Malaria at Karnal. Table XVII gives the rainfall, with the epidemic figures and indices of incidence (*vide* also Charts 6, 7 and 8). The relation between rainfall and malaria is that previously found in the Punjab (Christophers, 1911; Gill, 1920).

TABLE XVII.

Monthly rainfall and mean saturation deficiency, recorded at Karnal.
Rainfall in inches.

	1929	1930	1931	1932	1933	1934	1935
June ..	0.11	4.23	0.10	1.09	5.93	1.85	0
July ..	4.87	11.25	7.02	8.95	7.85	19.13	10.79
August ..	4.16	2.50	10.07	7.43	8.12	12.34	12.94
September ..	0	0.90	6.48	10.31	13.90	1.45	8.59
July and August	9.03	13.75	17.09	16.38	15.97	31.47	23.73
July to September	9.03	14.65	23.57	26.69	29.87	32.92	32.32
Epidemic figure	0.81	1.19	1.34	1.72	2.21	0.95	1.99
Index of incidence	14.0	55.8	12.3	58.7

Mean saturation deficiency.

Calculated from the first week in which the 4.0 p.m. saturation deficiency fell below 0.70 inch.

Mean of 4.0 p.m. readings.

	1929	1930	1931	1932	1933	1934	1935
First week ..	July 21	June 22	July 12	July 17	June 25	July 1	July 14
June	0.74	0.54
July ..	0.51	0.49	0.62	0.45	0.44	0.62	0.37
August ..	0.32	0.63	0.50	0.45	0.37	0.35	0.37
September ..	0.94	0.92	0.58	0.49	0.38	0.80	0.54
July and August	0.42	0.56	0.56	0.45	0.40	0.50	0.37
July to September	0.59	0.68	0.57	0.46	0.40	0.61	0.44

Mean of 4.0 p.m. and 8.0 a.m. readings.

	1929	1930	1931	1932	1933	1934	1935
July and August	0.34	0.43	0.38	0.31	0.27	0.34	0.27
July to September	0.46	0.51	0.38	0.31	0.27	0.40	0.31

When the July-August rainfall is deficient, there is little malaria. But when it is normal or excessive, there may or may not be an epidemic. It is the failure of an epidemic in years of higher rainfall which reduces the correlation between July-August rainfall and malaria.

Table XVII also shows the saturation deficiency for the same periods. We have used the saturation deficiency in place of relative humidity, because

CHART 6.

(1) Weekly means of temperature and saturation deficiency. (2) Weekly rainfall. (3) Epidemic figure (below number of year).

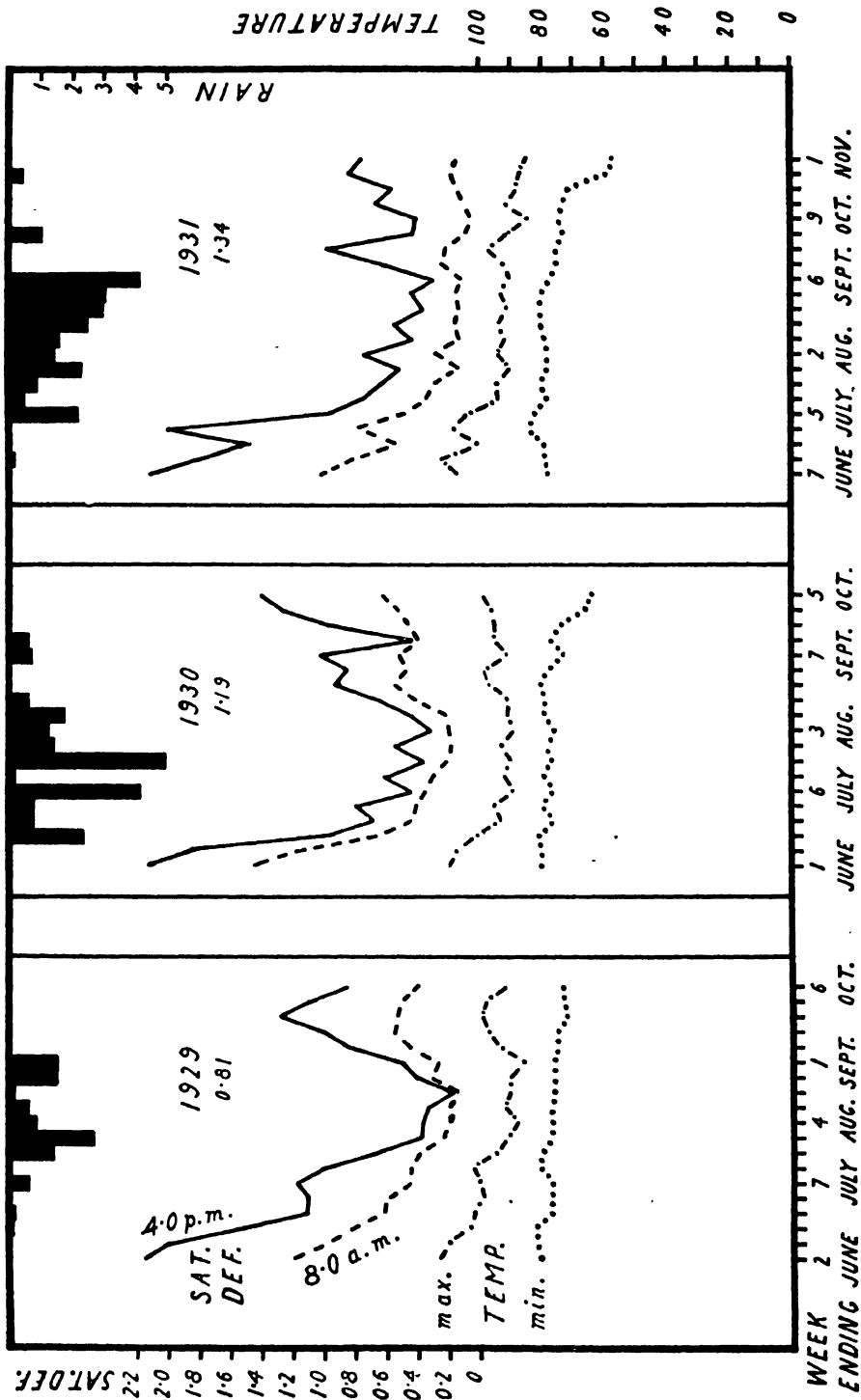


CHART 7.

- 1) Weekly means of temperature, saturation deficiency and catches of *A. culicifacies*. (2) Weekly rainfall.
- (3) Epidemic figure (below number of year). (4) First increase in cases of malignant tertian malaria (arrow).

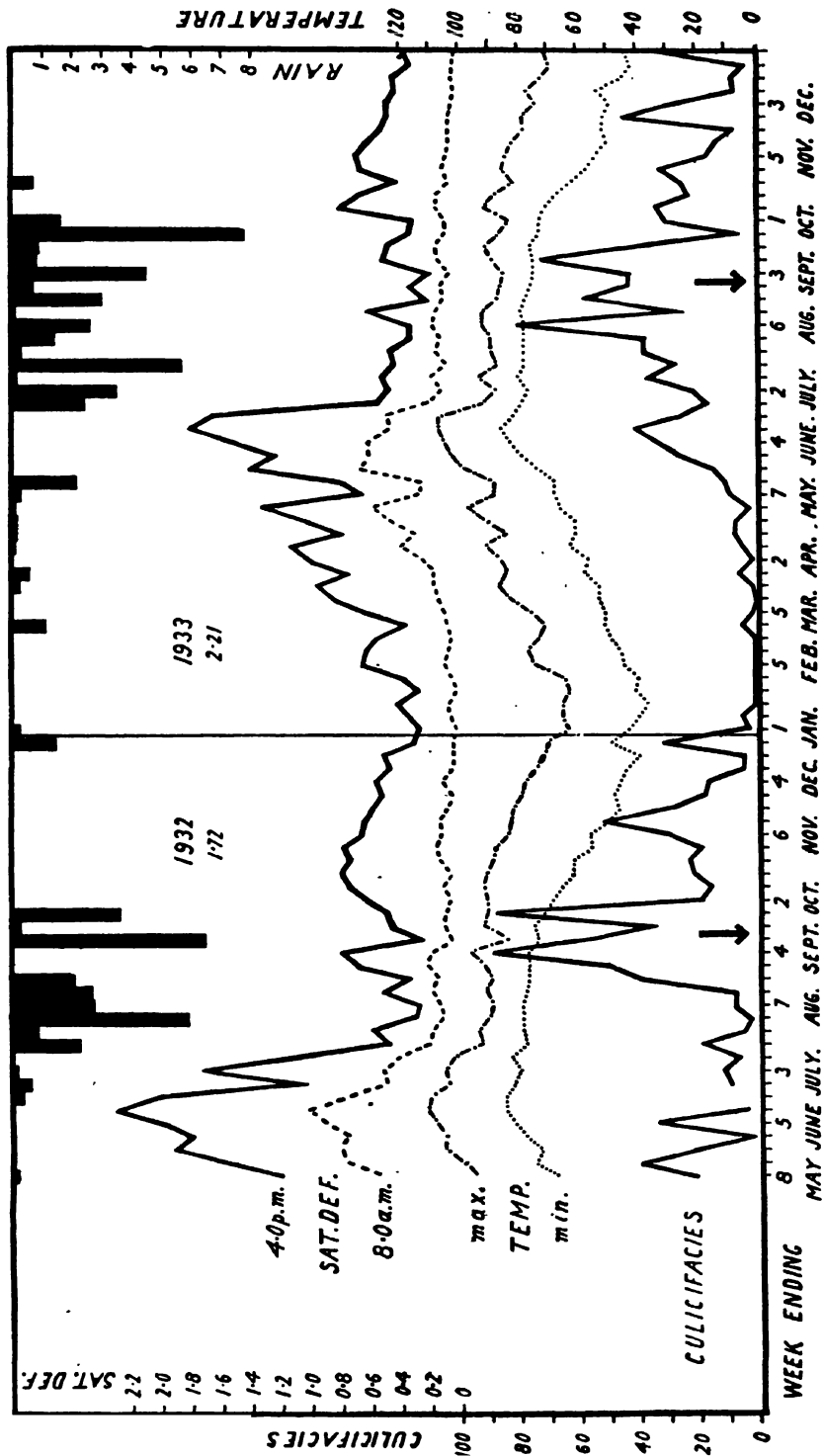
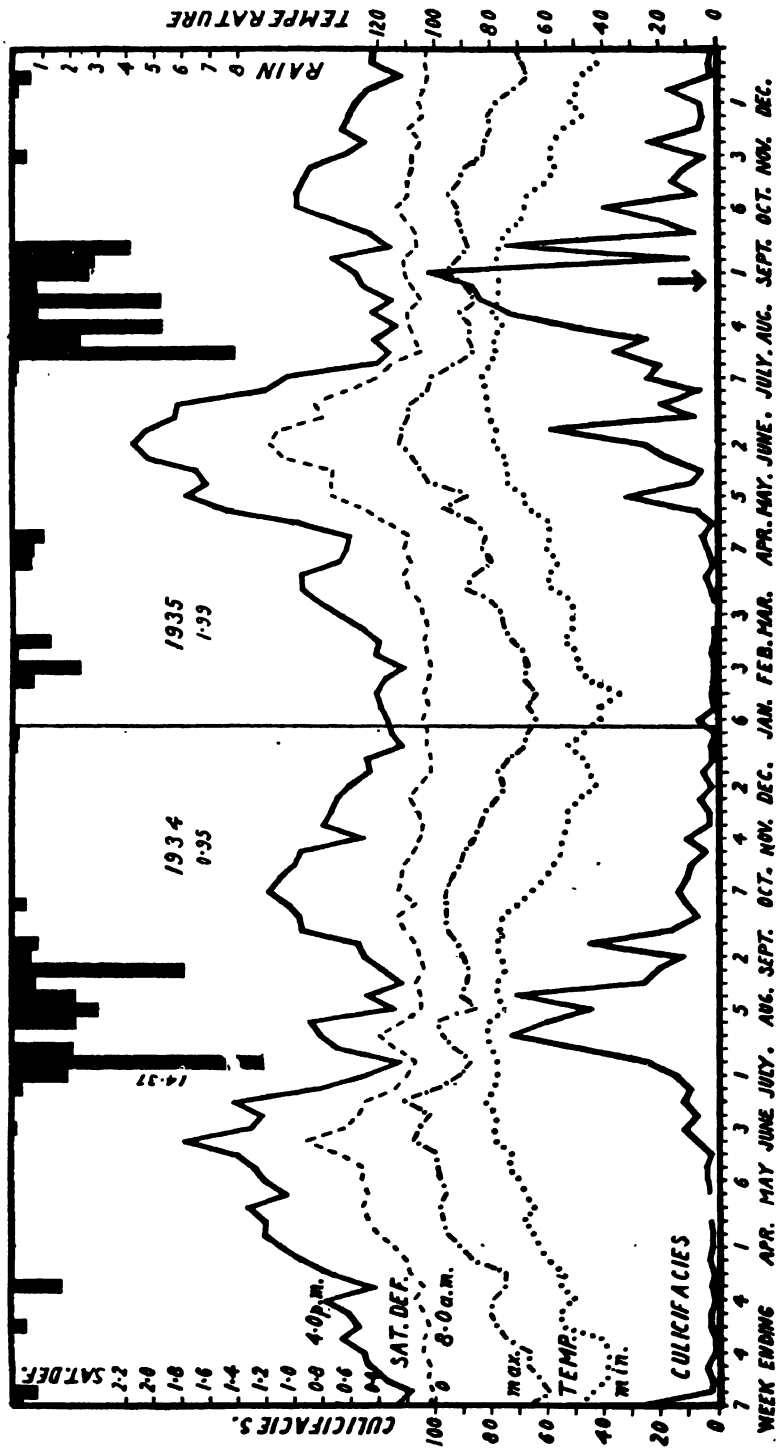


CHART 8.

(1) Weekly means of temperature, saturation deficiency and catches of *A. culicifacies*. (2) Weekly rainfall. (3) Epidemic figure (below number of year). (4) First increase in cases of malignant tertian malaria (arrow).



the former is a better index of the drying power of the air, which is believed to be an influence limiting the life of the mosquito. 'We may say that the loss of water from certain species of insects and their eggs is directly proportional to the saturation deficiency over a wide range of conditions. Others possess powers of controlling the loss to some extent.....But the fact that the loss of water is determined in the first instance by saturation deficiency and not by any other measure of humidity is of great importance. In analysing insect populations, whether in the laboratory or in the field, we now have an appropriate scale' (Buxton, 1933). The labour of calculating a large number of saturation deficiencies has been lightened by the construction of a nomogram, which is reproduced at the end of this paper. It is based on the vapour pressure tables of the Smithsonian Institution (1918).

The records of saturation deficiency agree more closely with the incidence of malaria. The morning saturation deficiency is low, and must nearly always be favourable to the mosquito. In the afternoon it is considerably higher, and shows the fluctuations more clearly. The two years of maximum incidence, 1933 and 1935, are the only years with two consecutive months having an afternoon saturation deficiency below 0.40 inch. In 1934, the year of highest rainfall but of low incidence, the afternoon saturation deficiency was low in one month only. A closer examination (Chart 8) shows that the heavy rainfall of July 1934 was chiefly due to 14.37 inches falling in the week ending on the 8th July. From the 10th to the 27th July the only fall was one of 0.04 inch. During this break the saturation deficiency rose. In 1932, a year of moderate incidence, there were a few well-separated showers in June (Chart 7), but the saturation deficiency did not fall till the week ending on the 17th July. At the end of August there was a break in the rains, and the saturation deficiency rose. In 1933 the rain began early and was evenly distributed over the whole of the transmission season. The saturation deficiency fell in the week ending on the 25th June and did not rise till October. The same sequence of events took place in 1935.

In the seven years of this survey the incidence of malaria is correlated less with the July-August or July-September rainfall than with the saturation deficiency. It seems to depend on the length of the period in which the saturation deficiency remained low, and this again depends on an even distribution of rainfall.

CLIMATE AND *A. CULICIFACIES*.

The graphs of catches of *A. culicifacies* (Charts 7 and 8) show wide fluctuations. Such fluctuations are shown in no other graph than that for the afternoon saturation deficiency. A rise in saturation deficiency is followed after one or two weeks by a fall in the catch of *A. culicifacies*. Table XVIII shows that this correlation is significant. After a high saturation deficiency, large catches were not made; after a low saturation deficiency the catches might be large or small. Some of these small catches following a low saturation deficiency were made early in the rainy season, before the number of mosquitoes had had time to increase. The correlation is closer than might have been expected, when one remembers that it is based on a rigid interval of two weeks, and that the actual catches may be influenced by such chances as a smoky fire in the room, wind or rain in the night, or the zeal of the collector. It may be objected that in dry weather the catches of *A. culicifacies*

TABLE XVIII.

Correlation between the saturation deficiency, measured at 4.0 p.m., and the number of female A. culicifacies caught two weeks later. Based on weekly means for the months of July to October in the years 1932 to 1935.

Saturation deficiency in inches.	NUMBER OF <i>A. culicifacies</i> .										
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109
0.2-0.29	..	1	1	2	1	1	2
0.3-0.39	1	1	2	1	1	2	..	1	1	..	1
0.4-0.49	1	2	3	3	1	2	1
0.5-0.59	4	1	..	1	..	1	..	1
0.6-0.69	1	1	1	1
0.7-0.79	2	1	2	1	1
0.8-0.89	1	..	1
0.9-0.99	..	4
1.0-1.09	1	1	..	1
1.1-1.19	2
and 1.70	..	1

Correlation coefficient, —0.3975; *P* less than 0.01; significant.

are lower because the mosquitoes retreat to damper and less accessible hiding places. But the charts show that the low catches were usually made two weeks after the dry weather, often at a time when the saturation deficiency had again fallen.

Another difficulty is that large catches were sometimes made in May and June, when the weather is very dry. They were made in the villages of Saidpura, Indri and Ghogripur, which are near the banks of the canal; never in those villages which are remote from it. Flooding or seepage from the canal may have caused a local fall in saturation deficiency.

It has been shown that, when the temperature is favourable, the length of life of a mosquito varies with the saturation deficiency. We therefore interpret the correlation between saturation deficiency and size of catch as due to the influence of saturation deficiency on length of life. If the life is shorter, there is not sufficient time for the development of sporozoites, and transmission is interrupted. Thus when the rainfall is actually deficient, the climate is never damp enough to allow serious transmission. When the rainfall is heavier and evenly distributed, there is a long period of low saturation deficiency, which is favourable to transmission. When the rainfall is heavy, but not evenly distributed, the breaks in the rain allow the saturation deficiency to rise and interrupt the cycle of transmission. Extensive and persistent flooding, of course, will also keep down the saturation deficiency, but this did not occur during our survey. On this view, the transmission of malaria in the Punjab is correlated with the length of the period of continuous low saturation deficiency.

We have not been able to separate the effect of temperature from that of saturation deficiency. It is known that high temperature may be fatal to insects, independently of the humidity (Buxton, 1933; Mayne, 1930; Freeborn, 1932). In the transmission season the temperature is not very variable, and

is obviously favourable. But in May and June temperatures of 110°F. and over are common.

Climate may affect transmission in another way. Mayne (1930) has shown that at certain temperatures and humidities *C. fatigans* is unwilling to bite. But a field survey naturally offers no evidence on this point.

Dose of infection.

The dose of infection depends on

- (1) the number of gametocyte carriers, and to some extent on the average gametocyte count,
- (2) the number of carrier mosquitoes,
- (3) the length of life of carrier mosquitoes,
- (4) the length of the transmission season.

In attempting to estimate the dose of infection we have used the percentage of crescent carriers. The average crescent count has been ignored. All crescent carriers who have been detected have at least ten, generally many more, crescents per c.mm. of blood, and we do not know the extent to which an increase in the average crescent count affects the transmission of malaria. The number of carrier mosquitoes is the mean for each month of all catches of *A. culicifacies*.

It is difficult to estimate the factor of longevity. It is to some extent reflected by the number of mosquitoes, because when the life is short the numbers will decline. The number of mosquitoes is also governed by the length of the transmission season, because when this begins early the catches in July will be higher. It would be possible to make some allowance for length of life by dividing the dose of infection by the mean saturation deficiency. But this is rather an artificial procedure, and we have preferred to calculate the dose of infection simply by multiplying the crescent rate by the mean catch of *A. culicifacies* for each month of July, August and September, and taking the mean of the products (Table XIX).

TABLE XIX.

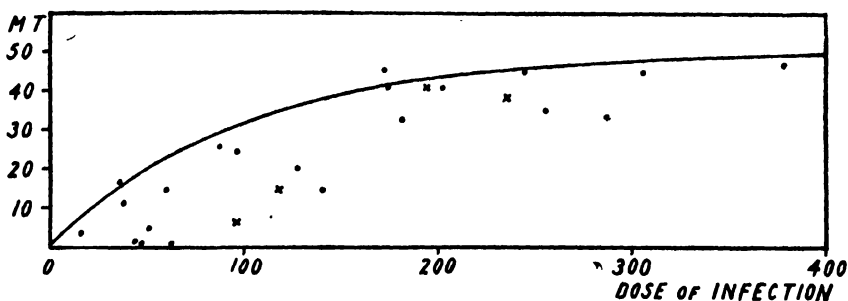
Calculation of dose of infection, i.e., product of crescent rate and mean catch of A. culicifacies.

	1932			1933			1934			1935		
	Crescent rate.	Mean catch of <i>A. culicifacies</i> .	Product.	Crescent rate.	Mean catch of <i>A. culicifacies</i> .	Product.	Crescent rate.	Mean catch of <i>A. culicifacies</i> .	Product.	Crescent rate.	Mean catch of <i>A. culicifacies</i> .	Product.
July ..	2.2	3.0	6.6	2.0	34.1	68.2	2.7	46.7	126	1.8	25.3	46
August ..	0.8	15.9	12.7	3.5	45.6	160.0	2.9	27.7	80	1.4	90.0	126
September ..	5.5	61.0	335.0	12.4	38.6	478.0	3.6	22.9	82	15.8	25.7	406
Dose of infection.	118	235	96	193

It will be seen that this estimate of the dose of infection is determined by the September crescent rate even more than by the catch of mosquitoes. In 1934, a year of low malaria incidence, the catch of mosquitoes is not low. But the September crescent rate is only 3.6 per cent, and is chiefly responsible for the low figure of the dose of infection. We believe that the low crescent rate is the result of defective transmission in July and August and that it may fairly be included as a factor in estimating the dose of infection. In support of this is the observation that the mean catch of *A. culicifacies* declined as the second progressed, showing that conditions were unfavourable to the mosquito.

CHART 9.

Dose of infection and number of new cases of malignant tertian malaria (MT). Dots, individual villages; crosses, combined villages. Period, malaria season each year, 1932 to 1935. The curve shows the theoretical relation, when 50 per cent of new infections are discovered.



The validity of this estimate of the dose of infection may be tested by comparing it with the observed number of new cases of malignant tertian malaria (Table XX and Chart 9). The latter has been calculated from the

TABLE XX.

Dose of infection for all villages combined. Index of incidence from spleen rate. Number of new cases of malignant tertian malaria among 100 children.

	Dose of infection.	Index of incidence.	New cases of malignant tertian.
1932	118	14.0	14.6
1933	235	55.8	38.5
1934	96	12.3	6.8
1935	193	58.7	41.0

parasite rate for asexual forms in the same way as the index of incidence was calculated from the spleen rate. It will be seen that this number does not rise directly with the increase in the dose of infection. This is to be expected.

Christophers (1915) has shown that if infections are distributed at random, the number of persons infected will follow the terms of a binomial expansion. Thus, if 10 infections are distributed among 100 persons, 9 will probably receive infections; if 100 are distributed, 63 persons will probably receive infections. It is possible to plot the number of infections distributed against the theoretical infection rate resulting. But it must be remembered that not all infections are discovered by the usual methods of examination. The curve of Chart 9 shows the relation between the number of infections distributed and the theoretical number of infections discovered, on the assumption that 50 per cent are discovered. It is at least not inconsistent with the distribution of the points already plotted. Our figures, in any case, cannot agree numerically with those of Christophers' theory, because our dose of infection is based on the mean catch of mosquitoes. It is a sample of the mosquito population, and we have no means of knowing the actual number of mosquitoes which bite each unit of the human population. Moreover, in the individual villages, the mean parasite rates are based on the examination of 100 to 250 children, and the mean mosquito catch on three searches only. The errors are therefore large.

Table XX shows that the dose of infection varies directly both with the index of incidence and with the number of new cases of malignant tertian malaria. Its variation is enough by itself to account for variations in the incidence of malaria.

EPIDEMIC AND ENDEMIC MALARIA.

The accepted views of the meaning and characteristics of endemic and epidemic malaria may perhaps be stated as follows. In endemic areas the intensity of malaria is on the whole constant from year to year. In epidemic areas the intensity is usually low, but occasionally flares up. For a given dose of infection, infants in both areas suffer equally. But the older children in an endemic area are better able to cope with infection because they are habituated to it, and the parasite rate declines steeply with increasing age. In an epidemic area the increase of immunity with age is less striking. The level of the

TABLE XXI.

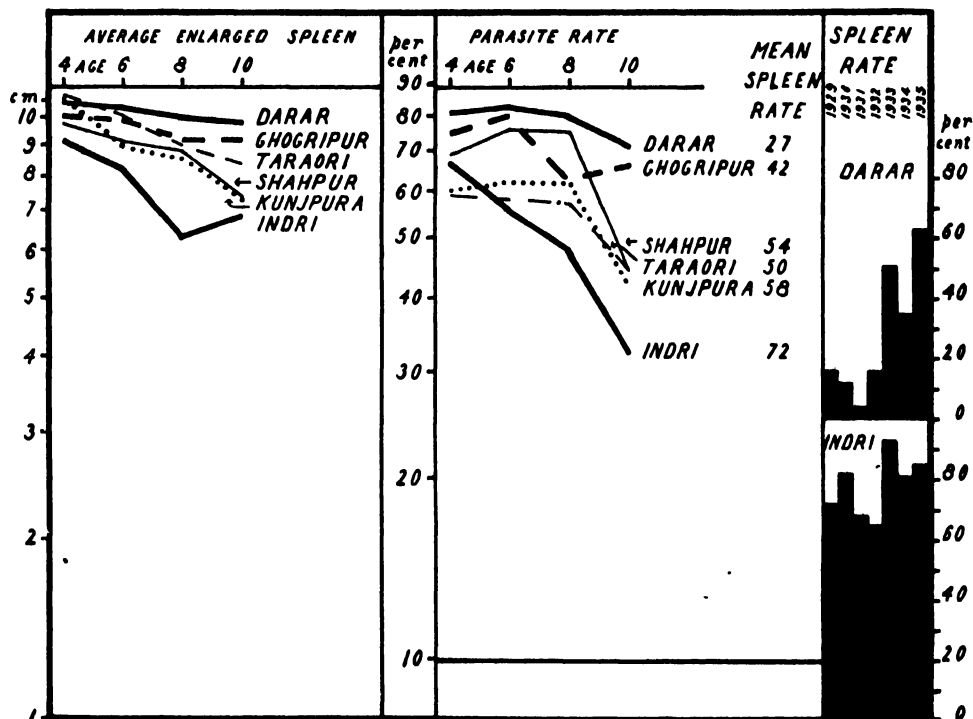
Individual villages. Mean average enlarged spleen and spleen rate, 1929 to 1936. Parasite rate in children with enlarged spleens, malaria seasons, 1932 to 1935. Mean catch of A. culicifacies, July to September, 1932 to 1935.

	Average enlarged spleen.	Coefficient of variation of AES.	Spleen rate.	Coefficient of variation of spleen rate.	Parasite rate in those with enlarged spleens.	Mean catch of <i>A. culicifacies</i> .
Darar ..	10.3	9.5	27	75	75	29
Ghogripur	9.7	9.2	42	44	71	29
Taraori ..	9.5	8.6	50	31	55	26
Shahpur ..	9.4	9.7	54	37	67	42
Kunjapura	8.7	10.0	58	35	59	45
Indri ..	8.0	9.3	72	18	47	50

parasite rate falls less steeply, and may even remain constant. This view may be examined in the light of the findings of our survey.

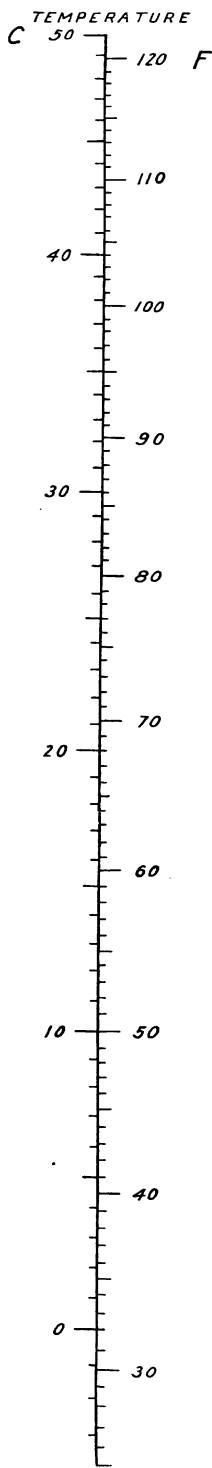
CHART 10.

Individual villages. Average enlarged spleen and parasite rate in children with enlarged spleens, by age groups, combined malaria seasons, 1932 to 1935. Mean spleen rate of each village, 1929 to 1936. Mean spleen rates of two villages, each malaria season, 1929 to 1935.

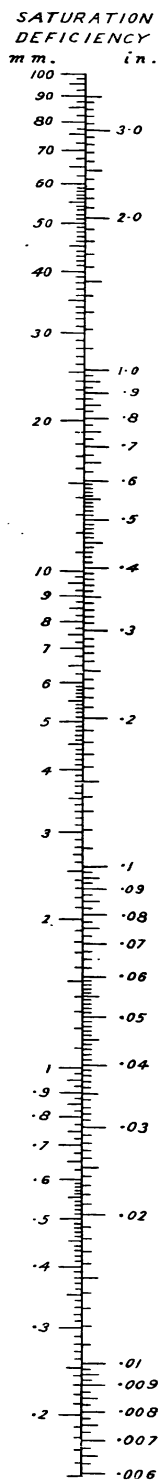


Certain statistics for individual villages are recorded in Table XXI and Chart 10. The mean spleen rate for Indri has a low coefficient of variation. The intensity of malaria has been constant, and is hyperendemic, since the rate is over fifty. In Darar the coefficient of variation is high, showing that the intensity of malaria has fluctuated widely. Darar is therefore subject to epidemic malaria. The other villages are intermediate.

For estimating immunity the parasite rate is unsuitable, because we do not know sufficiently accurately the dose of infection for each village. But we can assume that all those with enlarged spleens have been infected with malaria. We have therefore used the parasite rate in those with enlarged spleens. It has been shown that this rate falls with an increase in immunity and rises with an increase in infection. Since we can assume that in each village children of all ages received the same dose of infection, the gradient of this rate with increasing age will measure the relative increase of immunity in each village.



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RELATIVE HUMIDITY

per cent.



The curve for the epidemic village of Darar shows little change, while that for the endemic village of Indri shows a steep and consistent decline. The characteristic of the endemic village is a more rapid and more complete acquisition of immunity. In any country in which this characteristic is established, the parasite rates of different age groups may be used to determine whether the malaria is of the epidemic or endemic type.

Darar and Indri are less than nine miles apart. The difference in their malarial histories shows that the incidence of malaria may be independent of rainfall. Indri is on the bank of the Budha Khera Escape, near a swamp and the Western Jumna Canal. There is little permanent water close to Darar. The greater expanse of water near Indri may be responsible for its greater suffering from malaria, by providing more breeding places or by causing a local fall in saturation deficiency. We have no evidence to show the relative importance of these explanations.

SUMMARY AND CONCLUSIONS.

The yearly variation in the incidence of malaria has been described. The problem is to discover the extent to which it is due to variation in the degree of immunity, or of the dose of infection, or of both. For this purpose, an attempt has been made to set up measurements of immunity and infection.

It has been shown that, as usual, the older children are more immune. In the older children the figures for the parasite rates and counts are lower, the average enlarged spleen is larger, and the spleen rate in the off-season is higher. But, except in the case of the average enlarged spleen, the onset of infection in the malaria season evokes big changes in all of them, so that these rates are clearly determined by infection as well as by immunity. We have taken the average enlarged spleen as the best measurement of immunity, because its size increases steadily and consistently in those children who are more immune, and because it is little affected by changes in the dose of infection from season to season and from year to year.

The product of the crescent rate and the mean catch of *A. culicifacies* has been taken as the measurement of the dose of infection. It is not entirely satisfactory, because it does not allow full weight to the influence of climate on the longevity of the mosquito.

The fluctuations in the dose of infection are enough by themselves to account for the fluctuations in the incidence of malaria. We have found little evidence of any change in the immunity of the population in the period under review. If the size of the average enlarged spleen is accepted as a valid measurement of immunity, it shows that if there has been any change, it has been unimportant.

The immunity has been slowly and gradually acquired. That acquired by the older children seems to be less solid than that acquired by children of five or six who live in hyperendemic areas, where there is a constant high level of transmission. The immunity of the older children in the Karnal district is high enough to protect them from the worst effects of the malaria which is usually encountered there. When the level of infection is raised for a single year, as in 1935, the increase in immunity is small. A high level of infection, continued over several years, would be needed to confer an appreciably higher

immunity. It may be doubted if even a fulminant epidemic would have much effect in raising the immunity of the survivors. It would, of course, raise the level of immunity of the community, by destroying the youngest and most susceptible portion. But this would be replaced in two or three years.

We conclude that fluctuations in the malaria of the Karnal district are almost entirely due to fluctuations in the dose of infection, and that the dose of infection is determined chiefly by the length of the period of low saturation deficiency. The latter depends rather on an even distribution of rainfall than on the total amount of rain. Extensive flooding would also, no doubt, maintain a low saturation deficiency in the absence of an even distribution of rainfall. But this did not occur during the period of our survey.

ACKNOWLEDGMENT.

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APPENDIX I.

Spleen rate and average enlarged spleen (AES).

[illegible]

Sept.	..	78	5	8.8	47	17	6.3	51	62	7.7	78	14	7.8	40	20	8.6	79	39	9.1	373	25	8.2
Oct.	..	75	1	10.0	35	29	9.8	51	70	7.2	68	34	8.3	39	38	9.7	74	64	7.9	342	38	8.2
Nov.	..	60	7	10.0	7.2	68	56	8.0	42	62	..	78	76	8.1	248	51	8.2
Dec.	69	72	
Mean	9.4	7.6	
1932.																						
May	..	70	6	11.3	64	23	10.0	50	52	8.5	47	54	8.0	59	34	9.1	67	46	7.3	357	34	8.5
June	..	65	12	10.4	50	20	9.4	50	74	8.1	53	47	8.9	54	37	8.2	61	52	8.2	333	40	8.5
July	..	70	10	11.7	60	20	9.8	55	64	8.5	65	43	9.3	49	39	9.6	60	40	8.2	359	35	9.1
Aug.	..	81	7	10.7	50	24	10.3	65	63	9.3	66	36	8.8	65	37	11.0	72	50	9.5	399	36	9.6
Sept.	..	89	10	11.7	50	34	11.9	68	63	9.0	56	48	9.0	46	41	8.4	71	52	10.9	380	40	9.8
Oct.	..	78	22	10.0	54	33	9.4	48	73	8.2	65	62	9.6	39	64	8.7	60	65	10.8	344	51	9.6
Nov.	..	75	19	11.6	46	35	9.6	60	67	7.8	69	70	8.9	47	60	8.4	63	59	9.7	360	51	9.0
Dec.	..	80	11	11.0	58	22	8.8	52	58	6.5	59	46	7.6	54	56	8.8	55	62	10.1	358	40	8.5
Mean	11.1	9.9	8.2	8.8	9.0	9.9	9.1
1933.																						
Jan.	..	91	10	10.0	59	19	11.1	70	71	8.6	60	68	9.6	47	62	11.1	50	42	10.0	377	43	9.7
Feb.	..	78	13	10.1	80	16	10.1	46	76	8.9	59	58	8.4	47	28	8.7	52	42	9.2	362	35	9.0
March	..	79	11	11.0	60	20	9.7	57	74	8.0	77	65	9.0	42	36	9.4	54	26	9.7	369	39	9.0
April	..	80	14	12.0	49	10	9.4	48	67	8.6	74	53	7.2	40	40	11.2	55	33	10.9	346	35	9.2
May	..	76	11	11.6	60	23	10.9	50	64	9.1	57	69	7.8	57	30	10.6	69	33	10.5	369	36	9.5
June	..	80	9	10.3	56	25	11.2	50	58	9.0	80	50	8.2	49	33	10.6	51	29	9.6	366	33	9.3
July	..	77	12	11.6	60	17	11.1	63	48	7.8	50	52	7.9	40	40	10.3	67	16	10.1	357	29	9.1
Aug.	..	77	8	11.0	69	17	10.2	50	66	7.9	44	71	7.9	54	28	10.6	51	57	10.5	345	37	9.0
Sept.	..	72	28	10.7	60	32	10.3	51	84	8.2	55	76	8.0	36	53	9.5	56	68	9.2	330	55	9.0
Oct.	..	70	63	10.2	57	65	9.4	51	100	6.6	50	86	6.9	51	57	9.4	35	74	10.2	314	73	8.5
Nov.	..	80	53	10.1	67	54	9.1	60	98	6.5	74	72	7.9	33	64	10.1	41	83	9.2	355	69	8.5
Dec.	..	80	59	10.9	65	54	10.2	60	90	6.8	60	85	8.5	42	60	9.9	59	63	9.1	366	68	9.1
Mean	10.8	10.2	8.0	8.1	10.1	9.9	9.1

APPENDIX I—concl'd.

	DARAR.			GHOGRIPUR.			INDRI.			KUNJPURA.			SHAHPUTR.			TARAPORI.			COMBINED FIGURES.		
	Number of children.	Spleen rate.	AES.	Number of children.	Spleen rate.	AES.	Number of children.	Spleen rate.	AES.	Number of children.	Spleen rate.	AES.	Number of children.	Spleen rate.	AES.	Number of children.	Spleen rate.	AES.	Number of children.	Spleen rate.	AES.
1934.																					
Jan.	80	56	9.8	75	55	9.3	58	93	7.5	54	74	7.7	34	56	9.3	44	82	9.2	345	68	8.8
Feb.	71	55	10.3	45	69	9.8	51	98	7.4	61	81	8.2	32	47	8.8	45	76	8.6	305	72	8.7
March	66	50	10.6	60	53	10.1	60	93	7.7	60	75	7.4	35	60	9.0	47	62	9.3	328	66	8.8
April
May	60	57	9.5	71	44	10.4	63	83	7.5	65	89	8.0	49	41	10.2	59	66	9.9	367	64	8.9
June	80	44	10.2	30	54	9.3	60	95	7.6	73	64	9.2	40	58	9.9	39	64	9.6	372	62	9.1
July	70	44	10.4	56	39	10.9	35	94	7.2	48	79	8.0	49	51	10.0	41	59	9.9	299	58	9.2
Aug.	70	31	11.4	54	43	9.7	60	67	8.1	60	83	8.7	36	42	11.2	31	65	10.4	311	55	9.4
Sept.	45	40	11.9	71	51	9.6	47	75	9.1	49	82	10.8	53	49	9.9	69	58	10.1	334	58	10.0
Oct.	..	45	10.1	40	58	10.5	44	84	8.7	38	71	8.4	31	77	9.2	47	51	9.3	280	61	9.3
Nov.	76	25	9.8	58	52	9.9	54	83	8.2	60	78	9.9	37	76	9.3	47	57	9.3	332	59	9.3
Dec.	92	30	11.0	80	55	10.0	49	82	8.0	60	68	9.4	43	75	9.8	72	43	8.9	396	55	9.5
Mean	10.5	10.0	7.9	8.7	9.7	9.5	9.2
1935.																					
Jan.	80	29	11.2	61	48	9.6	44	89	9.5	73	60	9.6	44	73	9.8	49	47	9.9	351	54	9.8
Feb.	85	18	10.9	78	35	10.0	74	74	9.3	77	62	9.8	41	56	10.0	61	38	9.9	416	46	9.8
March	100	23	9.8	86	63	9.2	64	80	9.0	68	68	8.7	36	50	9.4	47	53	9.6	401	54	9.2
April	86	12	11.1	65	45	10.0	71	69	7.9	65	62	10.4	33	42	10.1	55	51	9.1	375	45	9.4
May	76	18	10.6	80	48	9.8	53	68	8.8	53	55	9.8	41	39	9.9	62	35	10.1	365	43	9.7
June	79	15	11.5	78	30	10.0	58	59	8.6	76	47	9.8	60	52	10.0	58	38	10.9	409	39	9.9
July	80	13	11.6	76	34	10.1	54	59	8.6	71	32	8.1	57	54	10.2	58	33	10.0	396	36	9.5
Aug.	80	21	10.2	80	40	9.7	70	54	8.5	64	41	8.7	66	29	9.8	69	28	10.0	429	35	9.4

Sept.	..	77	48	91	76	74	9-2	53	81	84	44	73	87	61	75	88	70	46	10-1	381	65	90
Oct.	..	80	66	93	35	83	9-5	72	85	7-2	73	69	84	48	90	83	44	70	89	352	76	85
Nov.	..	79	73	99	79	68	8-5	60	90	8-1	71	75	86	57	93	82	55	45	94	401	74	88
Dec.	..	80	63	100	82	65	9-5	72	85	7-8	50	84	85	38	95	82	60	62	88	382	73	88
Mean	10-4	9-6	8-5	9-1	9-4	9-7	9-3
1936.																						
Jan.	..	79	60	9-9	70	71	9-1	63	78	8-9	70	61	8-1	42	91	7-6	54	44	8-0	378	65	87
Feb.	..	79	65	9-5	79	63	8-7	41	81	8-1	77	57	9-0	45	89	8-6	52	42	9-2	373	64	89
March	..	71	63	10-3	80	55	9-6	70	79	8-0	71	62	8-5	40	78	7-5	47	43	8-9	379	64	88
April	..	59	58	10-1	75	67	8-9	60	78	8-7	80	60	8-1	50	78	8-6	50	48	9-3	374	65	89
Mean	10-0	9-1	8-4	8-4	8-1	8-9	88
1929-36.																						
Mean	..	27		10-3	42		9-7	72		8-0	58		8-7	54		9-4	50		9-5	50		9-0
St. dev.	..	20-5		0-98	18-3		0-88	18-2		0-74	20-4		0-87	19-6		0-91	15-8		0-81	14-7		0-49
Coeff. var.	..	75-2		9-5	44-0		9-2	18-3		9-3	35-0		10-0	36-6		9-7	31-3		8-6	29-5		5-5

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APPENDIX II.

Number of children examined. The ages shown are the upper limits of each age group. Pre-malaria season, May to August. Malaria season, September to December.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	55	227	442	389	325	58	281	433	365	303
1933	20	163	428	531	294	1	84	502	483	296
1934	4	107	410	458	370	35	191	408	424	284
1935	82	277	456	450	335	69	297	499	438	213
TOTAL	161	774	1,736	1,828	1,324	163	853	1,842	1,710	1,096

APPENDIX III.

Parasite rate for malignant tertian, asexual forms.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	7	10	9	8	7	26	17	23	26	19
1933	5	6	6	7	5	..	45	46	44	38
1934	..	21	18	21	20	20	25	24	23	20
1935	7	10	7	7	8	32	43	46	47	39
Four years.	7.5	10.4	10.1	10.9	10.5	27.0	30.8	35.5	36.0	28.2

APPENDIX IV.

Benign tertian parasite rate.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	15	14	10	11	8	14	25	18	12	7
1933	15	17	14	13	9	..	15	17	15	11
1934	..	16	16	11	7	9	18	10	11	7
1935	12	13	7	6	6	26	20	18	14	9
Four years.	13.7	14.5	11.9	10.2	7.3	17.8	20.6	15.9	12.9	8.8

APPENDIX V.

Crescent rate.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	4	3	3	2	0.6	5	3	8	10	7
1933	10	1	3	3	0.3	..	11	13	12	8
1934	..	3	4	4	4	6	4	4	4	2
1935	1	2	3	1	0.6	4	9	11	11	5
Four years.	3.1	2.2	3.1	2.5	1.5	5.5	6.2	9.2	9.1	5.8

APPENDIX VI.

Spleen rate.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	20	33	33	40	41	33	39	44	47	52
1933	15	28	33	36	36	..	70	65	65	69
1934	..	50	59	59	61	49	60	60	60	53
1935	18	39	33	38	47	41	70	75	74	74
Four years.	19.8	36.4	39.0	43.6	47.0	40.0	57.5	61.5	62.0	61.0

APPENDIX VII.

Average enlarged spleen.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	11.1	10.1	9.2	9.0	7.8	11.4	11.1	9.3	8.6	8.3
1933	..	11.5	10.0	8.8	8.4	..	10.0	9.6	8.3	7.8
1934	..	10.2	9.9	8.5	8.9	11.7	10.6	9.9	9.2	8.4
1935	11.5	10.5	10.0	9.6	8.6	10.9	9.7	8.8	8.4	7.7
Four years.	11.3	10.5	9.8	8.9	8.5	11.3	10.3	9.3	8.6	8.1

APPENDIX VIII.

Average parasite count, malignant tertian, asexual forms.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	..	425	5,600	75	575	44,500	4,600	5,400	2,050	925
1933	2,100	9,850	1,025	..	20,500	13,900	19,500	7,500
1934	..	1,925	1,900	890	650	..	2,275	2,625	1,775	1,300
1935	..	1,075	5,250	1,300	200	23,100	8,200	5,125	5,450	3,750
Four years.	150	3,975	3,425	2,400	600	27,200	8,250	7,850	9,200	4,150

APPENDIX IX.

Average parasite count, benign tertian.

	PRE-MALARIA SEASON.					MALARIA SEASON.				
	Age in years.					Age in years.				
	2	4	6	8	10	2	4	6	8	10
1932	..	792	588	1,874	522	..	1,892	1,780	588	125
1933	..	2,180	4,227	2,081	1,123	..	3,803	3,093	2,339	1,880
1934	..	1,096	1,448	1,294	653	..	2,439	1,289	1,439	819
1935	778	4,923	576	1,361	884	999	4,819	2,713	1,453	884
Four years.	500	2,480	1,940	1,725	790	1,070	3,160	2,370	1,570	1,060

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APPENDIX X.

Correlation of average enlarged spleen and spleen rate.

AES.	DARAE										GHOGRIPUR.					
	Spleen rate.										Spleen rate.					
	10 +	20 +	30 +	40 +	50 +	60 +	70 +	10 +	20 +	30 +	40 +	50 +	60 +	70 +	80 +	
60 +	1
65 +
70 +	1
75 +	1
80 +	1	1
85 +	1	1	1	3
90 +	1	1	..	1	..	1	1	1	1	3	2	2
95 +	1	2	2	2	1	..	3	1	4	3	2	..	1	..
100 +	6	2	..	3	3	3	..	2	2	4	2	3
105 +	2	1	2	2	1	..	1
110 +	7	1	2	2	1
115 +	7	1	1
120 +	1

Coeff. correl., — 0.308; P, 0.025.

Coeff. correl., — 0.136; P, 0.32.

APPENDIX X—contd.

AES.	TABAGORI.								SHANPUR.								
	Spleen rate.								Spleen rate.								
	10 +	20 +	30 +	40 +	50 +	60 +	70 +	80 +	10 +	20 +	30 +	40 +	50 +	60 +	70 +	80 +	90 +
6.0 +
6.5 +
7.0 +	1
7.5 +	1	1	..	1
8.0 +	3	1	..	1	1	1	..	2	3
8.5 +	2	..	1	2	3	..	1	1	1	2	1	..
9.0 +	1	4	4	3	..	2	2	..	4	1	2
9.5 +	..	2	1	1	4	2	1	3	1	2	1	2
10.0 +	1	3	3	2	1	2	1	3	3	4	1
10.5 +	3	..	2	1	1	2
11.0 +	1	2	..	1

Coeff. correl., — 0.293; *P*, 0.03.Coeff. correl., — 0.450; *P* less than 0.01.

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APPENDIX X—concl'd.

AES.	KUNJPURA.								INDRI.								
	Spleen rate.								Spleen rate.								
	10 +	20 +	30 +	40 +	50 +	60 +	70 +	80 +	10 +	20 +	30 +	40 +	50 +	60 +	70 +	80 +	90 +
6-0 +	1	..
6-5 +	1	1	3
7-0 +	1	..	1	1	..	4	2	2
7-5 +	1	1	1	1	3	2	1	7	..	2	3
8-0 +	2	..	3	3	3	2	2	2	4	5	1
8-5 +	1	2	..	2	3	3	5	4	4	1	..
9-0 +	1	1	..	2	1	3	1	3	2	1	..
9-5 +	..	1	..	1	1	4	1	1	..
10-0 +	1	1
10-5 +	..	1	1
11-0 +

Coeff. correl., — 0.243; *P*, 0.075.Coeff. correl., — 0.297; *P*, 0.015.

ON MALARIA TRANSMISSION IN THE JEYPORE HILLS.

Part I.

A YEAR'S DISSECTION RESULTS.

BY

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[19th October, 1936.]

THE Jeypore Hills, part of the Eastern Ghats Range, are situated approximately between $17^{\circ} 30'$ and 20° N., and $81^{\circ} 30'$ and 84° E. Their highest peak, Deomali, attains a height of 5,470 feet. The eastern edge of the massif lies roughly parallel to the Bay of Bengal, at a distance of about 30 to 100 miles from the coast, and generally the highest points are situated along the eastern escarpment, which rises abruptly from the plains. Behind these, the country is formed into a series of plateaux, with intervening scarps, at approximately 3,000, 2,000 and 1,000 feet levels, by which values they are generally known. The slopes of the hills are covered with poor 'Sal' forest (*Shorea robusta*), much damaged by shifting cultivation. The valley bottoms are more and more being opened up for rice cultivation.

The hills are inhabited by various aboriginal tribes, Khonds, Savaras, Gadabas, Porojas, etc., belonging to the Munda and Kolarian groups of pre-Dravidian races. Save in the inaccessible forests and side valleys they are in process of being absorbed through intermixture with the races of the neighbouring plains, Oriyas and Telugus, who are gradually immigrating into the hills as the warlike propensities of their original inhabitants are subdued by closer administration. Since 1839, when the hills first came under British administration, they have been administered under a special act by the Collector of Vizagapatam as Agent to the Governor of Madras; hence their alternative title, the 'Vizagapatam Agency Tracts', or, in local parlance, the 'Agency'. As from April 1936, they have been transferred to the new Province of Orissa, as a 'Partially Excluded Area', under the new Indian constitution.

CLIMATE.

There is no first class meteorological recording station in the hills. The climate is, to the European, generally pleasantly cool—in the winter months, distinctly cold. The writer has found it too cold when motoring on the 3,000-foot plateau in the rains. Though very hot in the day in April and May, the nights are always bearable. During these months, in the area covered by the present investigation, though a burning dry northerly wind blows by day from the scorched plains of Central India, at night this wind reverses and a cooler, moist air blows up from the coast. The *Gazetteer* (p. 146) gives the rainfall averages (shown in Table A) applicable to places which have been the subject of the present malaria investigations or of those of previous observers.

TABLE A.
Average rainfall figures.

Place.	Approx. elevation (in feet).	Period of record.	Jan. to March.	April and May.	June to Sept.	Oct. to Dec.	TOTAL.
Salur ..	570	1870-1904	1.40	4.39	26.83	10.96	43.58
Pottangi ..	2,800	1883-1904	1.60	6.08	44.81	10.12	62.70
Koraput ..	3,000	1877-1904	0.93	3.96	48.95	5.50	59.34
Jeypore town ..	1,920	1882-1904	0.94	4.18	64.61	5.40	75.13
Naurangpur ..	1,918	1882-1904	0.83	3.56	55.99	3.90	64.28
Malkangiri ..	641	1882-1904	0.56	3.08	58.12	4.70	66.46
Parvatipuram ..	395	1870-1904	1.90	4.55	30.98	8.69	46.12
Rayaghada ..	687 *	1877-1904	1.29	5.35	32.30	5.92	44.86
Bissamcuttack ..	1,114	1890-1904	1.59	5.92	36.46	4.06	48.03

* The height at the railway station, north of the town, is 712 feet.

From the time when they first became known to the British, these hills have been notorious for the intensity of the local malaria. It has been said 'to think of the Agency is to think of malaria'. The *Official Gazetteer* of the Vizagapatam district (Francis, 1907) states (p. 156) :—

'Malaria prevails throughout the whole of the Agency. The worst localities are perhaps the Bissamcuttack side, the Malkangiri taluk, and the Golconda Hills. The worst season of the year for the disease is undoubtedly in the rains, which is contrary to the usual rule in such matters.* The least unhealthy period is from November up to the first thunderstorms of April Blackwater fever is common among European residents in the hills. The hill people themselves seem to suffer little from malaria'.

PREVIOUS INVESTIGATIONS.

The first investigation into malaria in the Jeypore Hills was made by Stephens and Christophers (1902). They visited the hills in February (Christophers' personal communication), travelling up the Ghat road from Salur as far as Koraput on the 3,000-foot plateau only. They confined themselves to a survey of the anopheline species (figures on p. 29 of their paper), and a determination of spleen and blood indices (their Tables III, V and VI). They discovered *A. jeyporiensis* Theo., and associated the

hyperendemic conditions they encountered in the area with this species, and with *A. listoni* and *A. maculatus*. They did no dissections.

Perry (1914) spent some sixteen months in the hills in 1912-13, and made a very thorough investigation of the relationship of race to splenic and blood indices. He recorded fifteen species of *Anopheles* from the district, of which he collected six species in sufficient numbers for dissection. His results are given in the present paper as Table B.

TABLE B.

Results of the dissections carried out by Perry (1914).

Species.	Number dissected for gut.	Number dissected for gland.	Number infected.
<i>A. culicifacies</i> ..	359	279	0
<i>A. listoni</i> ..	52	268	4
<i>A. jeyporiensis</i> ..	61	402	0
<i>A. theobaldi</i> ..	1	1	0
<i>A. maculipalpis</i> ..	12	19	0
<i>A. fuliginosus</i> ..	8	13	0

The only infections found were in *A. listoni*. Details are not given, but from p. 485 of his paper it is clear that Perry found sporozoites in the glands of this species—under which name he apparently included *A. fluviatilis*, *A. varuna* (not then differentiated) and *A. minimus*.

Perry's dissection work was done on the 3,000-foot plateau, probably around Koraput, and it is interesting to note the very low infection rate he found compared with those of the present author around the 1,000 feet level. Perry's infected specimens were all taken in September to November. In the period March to August he found no infections. He seems to have done no dissections in December to February.

Perry's Map I shows the locations of the places he examined, all to the west of the escarpment of the 3,000-foot plateau. Of the three areas mentioned by the *Gazetteer* as being the most malarious in all these hills, he did not visit the Golconda taluk, in the south of the 3,000-foot plateau, nor the Bissamecuttack 'side' of the district. It is probable that transport difficulties were responsible for these omissions, for the very mountainous Golconda taluk is to this day without road communication, and the 'road' from the plains to the Bissamecuttack area after the first 35 miles had little existence, except on the Ordnance map, as late as 1925.

Thus the entire eastern third of these hills was malariologically unknown, until 1925. That it was intensely malarious was a matter of common knowledge, but it was left blank in the Malaria Map of India of Christophers and Sinton (1926), under the caption 'probably hyperendemic hill areas'.

This eastern third of the hills is, geographically, rather dissimilar to the rest of the massif. Broadly, it consists of a gradually rising and narrowing valley, bounded on the west by the escarpment of the 3,000-foot plateau, and on the east by the Savara Hills, rising from some 400 feet elevation at

Parvatipuram to 1,350 feet in the pass at Chatikona. Beyond this the Bissamcuttack plateau opens out with an average elevation of about 1,200 feet, running to the frontier of Kalahandi Feudatory State, through which it gradually slopes northward down to the Tel River, a main tributary of the Mahanadi. In the north-western corner the Ningiri group of hills rises to nearly 5,000 feet. The inhabitants are nearly all of Khond blood, semi-civilised in the valley, 'wild' in the hills themselves. Referring to the small town of Bissameuttack, the headquarters of the taluk of that name, the *Gazetteer* states (p. 232):—'The name means "poisonous fort" and is usually supposed to have been earned by the virulence of the malaria there, which is a byword throughout the district'. These are strong words for an official publication.

It was through this country that the Raipur-Vizianagram Railway was constructed between 1925 and 1931, the present author being in charge of the anti-malaria operations involved. A preliminary account of the country and the protection work have been published (Senior White, 1928). Spleen indices were given for many villages along the route, some parasite findings were recorded, and two other species of anophelines, *A. maculatus* and *A. vagus*, were added to Perry's list. No dissection work was done and malaria control was achieved by attacking all species found that were then proved carriers somewhere in Asia, viz., *A. culicifacies*, *A. funestus*, *A. maculatus* and *A. theobaldi*. The latter was attacked, not as a proved carrier, but on account of its exceedingly close systematic relationship to *A. maculatus*. Under *A. funestus* were included all the three sub-species, *A. fluviatilis*, *A. varuna* and *A. minimus*.

Protection work on the Agency section of the Raipur-Vizianagram Railway has continued uninterruptedly; although, after its opening for traffic, it was confined to the stations, eight in number, located on its length. The efficiency of the anti-larval work is tested by routine weekly adult catches, hence a considerable mass of data is now available.

The anopheline faunal list for the area now stands as under :—

<i>A. aitkeni</i> James.	<i>A. maculatus</i> Theobald.
<i>A. hyrcanus nigerrimus</i> Giles.	<i>A. theobaldi</i> Giles.
<i>A. barbirostris</i> v. d. Wulp.	<i>A. karwari</i> James.
<i>A. subpictus</i> Grassi.	<i>A. majidi</i> McCombie Young and Majid.
<i>A. vagus</i> Dönitz.	<i>A. stephensi</i> Liston.
<i>A. culicifacies</i> Giles.	<i>A. splendidus</i> Koidzumi.
<i>A. fluviatilis</i> James.	<i>A. tessellatus</i> Theobald.
<i>A. varuna</i> Iyengar.	<i>A. jamesi</i> Theobald.
<i>A. minimus</i> Theobald.	<i>A. annularis</i> v. d. Wulp.
<i>A. aconitus</i> Dönitz.	<i>A. philippinensis</i> † Ludlow.
<i>A. jeyporiensis</i> * James.	<i>A. pallidus</i> Theobald.
<i>A. moghulensis</i> Christophers.	

* Specimens resembling the *candidiensis* form have been seen on a few occasions.

† Identification on larvæ and a single ♂ only. Requires confirmation.

These 23 species include all those recorded in earlier publications, and the list is not likely to be increased, unless exploration of the very inaccessible higher peaks reveals the presence of the montane species *gigas* and *lindesayi*.

Numerically, the fauna is dominated by the following species:—*culicifacies*, *funestus* group, *jeyporiensis*, and *subpictus-vagus*, though probably *maculatus* and *theobaldi* are more numerous than adult records indicate, judging from the numerous larvæ of these species which are to be found in suitable streams and in scēpages.

Some interesting facts regarding seasonal distribution emerge from the records. Two years' weekly catches, carried out for 20 minutes at a time in four cattle sheds in and around the periphery of the protection on Chatikona Summit Station*, have produced the monthly figures shown in Table I for the three dominant species of importance.

TABLE I.

Monthly ♀ catch in four cattle sheds in villages near Chatikona Summit.

Month.	1934-35.			1935-36.		
	<i>A. culicifacies</i> .	<i>A. funestus</i> †.	<i>A. jeyporiensis</i> .	<i>A. culicifacies</i> .	<i>A. funestus</i> †.	<i>A. jeyporiensis</i> .
June ..	321	3	2	140	0	0
July ..	262	0	0	391	0	0
August ..	208	2	2	193	0	0
September ..	242	3	8	139	1	0
October ..	52	1	0	37	8	21
November ..	5	20	149	13	21	79
December ..	0	32	279	15	31	203
January ..	2	5	176	42	20	140
February ..	26	10	154	38	5	98
March ..	33	4	122	87	5	60
April ..	58	0	49	102	6	14
May ..	71	0	10	166	0	3
TOTALS ..	1,280	80	951	1,363	97	618
PERCENTAGES	57.9	3.6	38.5	65.6	4.7	29.7

* Recently re-named 'Bissamcuttack', though 5 miles distant from the town of that name. As the numerous malariologists who have visited the section with me have all known the station under its former name, which is that of the village close by, and as the town has a separate existence in the literature of malaria, the old name of the station is retained for purposes of publication.

† In this paper '*A. funestus*' includes *A. fluviatilis*, *A. varuna* and *A. minimus*. The reasons for including these species under the group name of '*funestus*' are given subsequently in this paper (*vide p. 67*).

For comparison with the same species in human habitations, the only figures available are those of catches in the same villages made during the past year for dissection purposes (including specimens not dissected because dead and dry). These are given in Table II, which is arranged in the same monthly order as Table I, for facility of comparison.

TABLE II.

Monthly ♀ catch in houses in four villages near Chatikona Summit.

Month.	<i>A. culicifacies.</i>	<i>A. funestus</i> *.	<i>A. jeyporiensis.</i>
June 1936 ..	145	21	5
July " ..	149	26	2
August " ..	135	66	0
September " ..	49	166	0
October 1935 ..	16	84	11
November " ..	4	295	42
December " ..	16	141	2
January 1936 ..	17	184	28
February " ..	29	100	21
March " ..	52	36	14
April " ..	129	29	14
May " ..	150	22	11
TOTALS ..	891	1,170	150
PERCENTAGES ..	40.3	52.9	6.8

Some very interesting facts emerge. *A. funestus** has its maximal incidence from September to February. It is dominantly attracted to human habitations, and only overflows into cattle sheds in the peak months.

A. culicifacies distinctly prefers cattle sheds to houses as a resting place. It is much less common in the cold weather than in the spring and the rains.

A. jeyporiensis has somewhat dissimilar attributes to *A. culicifacies*. Whilst it obviously prefers cattle sheds to houses as resting places, it has its peak of prevalence in November to March. During August and September, the months of highest rainfall, it disappears altogether.

The month of October, in which the three species are all undergoing crises of increase or decrease, is peculiar, inasmuch as in that month their breeding is in each case minimal. The vernal change-over of species is not marked by minimal breeding. Some quite unknown, but profound, cause must therefore be operative in the month of October.

* In this paper '*A. funestus*' includes *A. fluviatilis*, *A. varuna* and *A. minimus*. The reasons for including these species under the group name of '*funestus*' are given subsequently in this paper (*vide p. 67*).

Perry (1914) made similar observations between August and October, on the 3,000-foot plateau, which yield corroborative evidence. Combining the figures on pp. 484 and 485 of his paper we get :—

In houses.			In cattle sheds.		
*C.	F.	J.	*C.	F.	J.
43	324	115	1,976	88	43

*C. = *A. culicifacies*; F. = '*funestus*'; J. = *A. jeyporiensis*.

Abstracting from Tables I and II of the present paper the figures for the corresponding months to Perry's observations, we get the following percentages for both sets of figures :—

	In houses.			In cattle sheds.		
	*C.	F.	J.	*C.	F.	J.
Perry, 3,000 feet	8.9	67.2	23.9	93.8	4.2	2.0
Senior White, 1,000 feet	37.9	59.9	2.2	93.5	2.1	4.4

*C. = *A. culicifacies*; F. = '*funestus*'; J. = *A. jeyporiensis*.

The resemblance of the results on the 3,000-foot and 1,000-foot plateaux is very close for all species in cattle sheds, and for '*funestus*' in houses. But on the higher plateau far fewer *A. culicifacies* and many more *A. jeyporiensis* are found in houses. This may be a climatic effect, or due to differences in the construction of the houses of the Gadabas and Porojas on the 3,000-foot plateau and of the Khonds on the 1,000-foot plateau.

During the construction of the Raipur-Vizianagram Line and as a result of experience gained subsequently in maintaining malaria protections, an impression was formed that the malaria potentialities of '*funestus*' were much greater than those of *A. culicifacies*. 'The nearer the hills the more the malaria' became a watchword with the medical staff of the construction. This is well brought out by the spleen census results shown in Plate II (Senior White, 1928), relating to the first forty miles into the hills from Parvatipuram. In this situation a good deal of open country is met with where the railway is more than half a mile from the hill foot, i.e., away from the principal '*funestus*' breeding grounds. Practical use was made of this empirical experience in siting Rupra Road Station, in Kalahandi Feudatory State, immediately north of the length dealt with in this paper. This was deliberately located over three-quarters of a mile from the river which was the nearest '*funestus*' breeding place, with the result that though the station has, on more than one occasion, been examined and found full of *A. culicifacies*, malaria has never been a problem there. None-the-less, we had no evidence in support of the belief that this species took no part in the transmission of malaria in this district, but only sufficient to make us think that it was a less efficient carrier. The true facts have only now emerged when, with the malaria section of the Medical Department of this railway organized and working smoothly, time has been found from routine duties for a certain amount of laboratory investigations.

This work now to be recorded was begun with a purely utilitarian object. It had all along been assumed that (a) at least '*A. funestus*' and *A. culicifacies* were both carrying, and (b) that transmission was perennial. Budgets, and work programmes, for the section, had been framed accordingly. In September

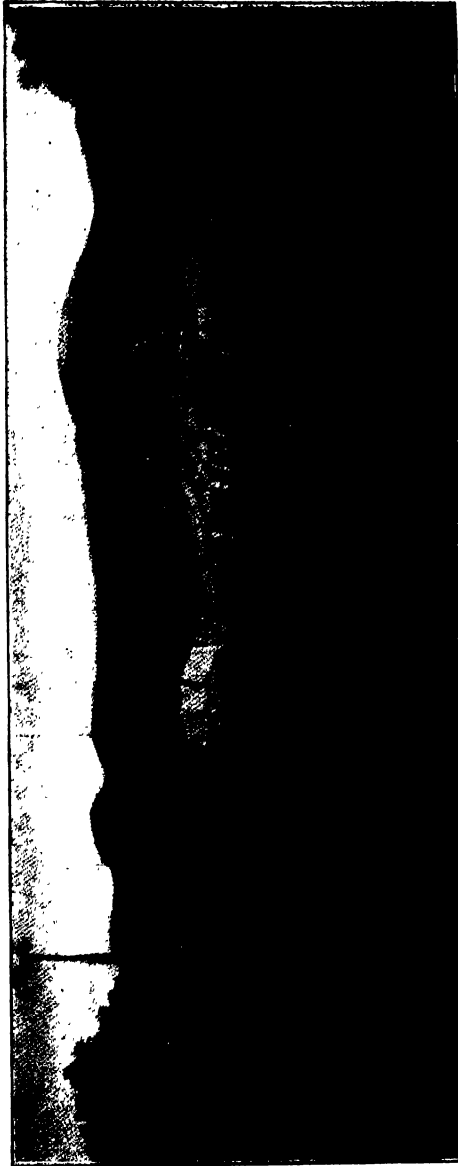
1935, a year's dissection programme was commenced to test these assumptions, using as the test area the hill section, situated between miles 155 and 200 from Raipur, which is the most intensely malarious length. The villages mentioned in Table C, hardly, or not at all, affected by the anopheline control measures in force around the stations, were chosen, and the inspectors in charge of the protections were instructed to make monthly catches and despatch them to headquarters in Calcutta for dissection. Villages within reasonable walking distance of stations had to be selected, so as not to take the inspectors too long off their regular work. They were instructed generally to catch in human habitations, unless otherwise advised; but with certain exceptions, unfortunately, the villages available often have the animals living in the same buildings as the human beings. This is particularly the case with villages inhabited by Doms ('Dambos' of Perry's paper), where conditions are further complicated by the presence of pigs, which none of the other castes keep. Details regarding the selected villages are given in Table C.

TABLE C.

Nearest station.	Village.	Approx. elevation (in feet).	Inhabitants.	Spleen rate*.	REMARKS.
Theruvalli Chatikona Summit.	Polypinda Coolie camp	892	Khond with Telugu blood. Mixed plains races.	50. February 1928 90. August 1936 ?	½ mile within periphery of protection. Shifting population.
"	Bariguda	1,363	Khond, etc.	70. October 1927	On actual periphery.
"	Jimidiguda	"	"	93. September 1936 89. April 1936	On actual periphery.
"	Matkabadi	"	Khond and Dom.	81. January 1928 100. March 1935 62. October 1927	Within periphery of protection.
Muniguda	Tikarapara	1,165	"	84. August 1936 61. October 1927	On actual periphery.
"	Monikol	"	Khond	83. August 1936 70. December 1927 97. March 1935 82. August 1936	At protection centre. Shifting population (for comparison with outside conditions).
Ambodala	Ambodala	1,214	Dom	91. August 1936	On periphery.
Lanjigarh Road.	Coolie camp	996	Mixed plains races.		
"	Kakbhata	"	Khond	97. March 1935 81. August 1936	

* The general rise in the village spleen rates is noteworthy. In 1927-28 earthwork had not been commenced. I fear the phenomenon is one of 'man-made' malaria connected with borrow-pits, or possibly with the introduction of foreign strains of the parasites.

All these are within the Agency except the last two, which are about $1\frac{1}{2}$ miles north of the frontier, within Kalahandi Feudatory State.



Jimidiguda village—showing the two lines of houses and the communal cattle sheds in the centre.
A typical Khond village, except that the panther-fence does not completely enclose the whole settlement.
Escarpment of the 3,000-foot plateau in the background.

As a brief experience served to show that infected specimens were never found in cattle sheds, searching such, specifically, was abandoned, save in the case of Jimidiguda village, where the cattle sheds are entirely separate from

the houses, being situated in a row between the houses, which are built in two long lines, facing inwards to the sheds. Any mosquito found in the cattle sheds in this village, unless it has approached from either end of the line of houses, must have passed deliberately over human blood to attack cattle.

None-the-less, to make up numbers, cattle sheds combined with houses have at times been searched, as indicated in the headings to Tables III to XII.

Again, here and there a month will be found blank. The reason for this is that Khonds are not altogether a race to take liberties with. There are times when they simply will not admit strangers, however familiar to them, into their villages. Throughout these hills there occurs, in April and May, a festival, the 'Chaitra Parvam', which is a true Saturnalia, when the villages are specially difficult of approach. 'Balli Jatra', a fire-walking ceremony, occurs in September and October, and the effect of both of these can be seen in Table XII relating to Kakhbata village, which being in an Indian State, is even more than usually independent in its attitude. At Muniguda, one or other, or both villages, were searched for anophelines on the same day of each month. They are therefore combined into one table.

The tables do not include all species taken in the catches. Obvious non-carriers, like *A. subpictus* and *A. vagus*, are excluded. *A. annularis* also is not tabulated. In my paper of 1928 (p. 14) I have given reasons for neglecting this species as a carrier in these hills. During the present investigation six specimens only were dissected, all negative.

Every specimen was dissected both for gut and gland infection, using the 'brilliant cresyl blue (0.05 per cent) saline' of Giovannola (1934), which I have found very valuable in bringing out oöcysts at first glance. Sporozoites are not stained, but show up clearly against the very faintly tinted saline. All dissections were made 48 to 72 hours after capture, i.e., as soon as the stomach contents were digested. Naturally a few mishaps occurred, whereby one organ or the other was lost, especially during the hot weather, when considerable mortality occurred and dead specimens had to be dissected at times, as numbers in any case were low. Of the species found positive during the work the percentages lost are shown in Table D.

TABLE D.

Species.	Gut.	Glands.
<i>A. culicifacies</i> ..	1.2	0.7
<i>A. fluviatilis</i> ..	1.1	1.6
<i>A. varuna</i> ..	1.6	0.6
<i>A. minimus</i> ..	1.0	2.0
<i>A. aconitus</i> ..	2.8	1.9
<i>A. jeyporiensis</i> ..	1.2	1.9

The figures given in Table D are too low appreciably to affect infection rates worked out from the total numbers, which, therefore, are tabulated without correction, thus simplifying the tables.

TABLE III.
Polypinda village, house catch, some cousheds also included.

Month, 1935-36.	A. culicifacies.			A. fluviatilis.			A. varuna.			A. minimus.			A. jeyporiensis.			A. maculatus.			A. pallidus.		
	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +
October ..	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
November ..	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
December ..	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
January ..	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
February ..	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
March ..	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
April ..	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May ..	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
June ..	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
July ..	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
August ..	60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
September ..	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTALS ..	618	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rate per cent.
Percentage of catch dissected.	97.8	1.4	0.4	0.2	0.2

G = Gut.

Gl = Salivary gland.

TABLE IV.
Chatikona contractor's camp : house catch.

Month, 1935-36.	<i>A. culicifacies.</i>			<i>A. fluviatilis.</i>			<i>A. varuna.</i>			<i>A. minimus.</i>			<i>A. aconitus.</i>			<i>A. jeyporiensis.</i>		
	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +	No.	G +	Gl +
October ..	12	0	0	14	4	1	1	0	0	1	0	0	1	0	0	1	0	0
November (2) ..	1	0	0	83	4	3	11	1	0	0	1	0	2	0	0	6	0	0
December ..	5	0	0	12	0	0	0	0	0	0	0	0	3	0	0
January ..	2	0	0	26	1	0	4	0	0	0	0	0	4	0	0
February ..	7	0	0	11	0	0	2	0	0	0	0	0	2	0	0
March ..	32	0	0	3	1	0	2	0	0	1	0	0	5	0	0
April ..	48	0	0	5	0	0	3	0	0
May ..	17	0	0	1	0	0
June ..	45	1	0	1	0	0	2	2	1
July ..	41	0	0	10	0	1	2	0	0
August ..	31	0	0	8	1	1	2	0	0
September * ..	42	0	0	27	3	1	6	1	0
TOTALS ..	283	1	0	200	14	7	56	4	1	24	1	0	3	0	0	22	0	0
Rate per cent	0.4	0	..	7.0	3.5	..	11.1	2.8	..	4.2	0
Percentage of catch dissected.	49.8	35.2	6.3	4.2	0.6	3.9

G = Gut. Gl = Salivary gland.

* September 1935, included.

TABLE V.
Jimidiguda village : house catch.

Month, 1935-36.	<i>A. culicifacies.</i>		<i>A. fluviatilis.</i>		<i>A. varuna.</i>		<i>A. minimus.</i>		<i>A. aconitus.</i>		<i>A. jeyporiensis.</i>		<i>A. splendidus.</i>		<i>A. theobaldi.</i>		<i>A. pallidus.</i>	
	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +
October ..	2	0	15	2	0	1	0	0	..	0	0	0	..
November	33	5	0	13	0	0	..	0	0	0	..
December ..	4	0	56	0	0	7	0	0	2	0	..	0
January	32	4	0	8	1	0	2	1	..	0
February ..	1	0	10	0	0	4	2	0	0	0	1	0	..	0
March ..	4	0	..	0	..	1	0	0
April ..	2	0	2	0	0	..	1	0
May ..	44	0	..	0	..	0	0	1	0	..	0
June ..	28	0	5	0	0	3	1	0	2	0
July ..	38	0	3	0	0	0	0	0
August ..	45	0	5	0	0	2	0
September ..	48	1	3	0	0	1	0	0
September ..	8	0	18	1	0	7	0	0
Totals ..	220	1	184	12	6	52	5	2	31	1	0	6	0	0	8	1	0	0
Rate per cent.	..	0.5	..	6.5	3.3	..	9.6	3.8	..	3.2	0	12.5	0
Percentage of catch dissected.	43.4	..	36.3	10.3	6.1	0.2	1.5	..	0.2	..

G = Gut.

Gl = Salivary gland.

TABLE VI.

Jimidiguda village: cattle shed catch.

Month, 1935.	<i>A. culicifacies.</i>		<i>A. fluviatilis.</i>		<i>A. varuna.</i>		<i>A. minimus.</i>		<i>A. aconitus.</i>		<i>A. jeyporensis.</i>		<i>A. splendidus.</i>		<i>A. maculatus.</i>		<i>A. theobaldi.</i>		<i>A. pallidus.</i>		
	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	
October ..	20	0	0	5	0	0	0	0	
November ..	4	0	0	0	16	0	0	0	0	
December	7	0	0	3	0	0	0	0	
January ..	2	0	0	0	1	0	0	..	1	0	0	0	0	
February ..	17	0	0	
March ..	1	0	0	
April	
May ..	37	0	0	
June ..	35	0	0	2	0	
July ..	62	0	0	0	0	
August ..	48	0	0	
September ..	36	0	0	1	0	
Totals ..	262	0	0	10	0	0	2	0	0	25	0	0	119	0	0	6	0	1	0
Rate per cent.	
Percentage of catch dissected.	60.5	2.3	0.5	5.8	27.4	1.4	..	0.2	..

G = Gut.

Gl = Salivary gland.

TABLE VII.

Bariguda village : house catch.

Month, 1935.	<i>A. culicifacies.</i>		<i>A. fuvialis.</i>		<i>A. varuna.</i>		<i>A. minimus.</i>		<i>A. aconitus.</i>		<i>A. jeyporensis.</i>		<i>A. splendens.</i>		<i>A. theobaldi.</i>		<i>A. pallidus.</i>	
	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +
October ..	1	0	9	1	..	3	1	17	4	1	..	0	..	0	..	0	..	0
November	36	3	8	0	0	..	0	..	0	..	0	..	0
December
January ..	2	0	13	1	..	4	0	1	0	0	..	0	..	0	..	0	..	0
February ..	11	0	15	3	2	18	4	4	0	0	..	0	..	0	..	0	..	0
March ..	13	0	5	0	0	7	1	4	1	0	..	0	..	0	..	0	..	0
April ..	10	0	10	0	0	4	0	5	0	1	..	0	..	0	..	0	..	0
May ..	35	0	3	1	0	3	1	2	0	0	..	0	..	0	..	0	..	0
June ..	37	0	3	0	0	1	0	1	0	0	..	0	..	0	..	0	..	0
July ..	50	0	3	0	0	3	1	1	0	0	..	0	..	0	..	0	..	0
August ..	41	0	25	4	3	11	1	14	0	2	..	0	..	0	..	0	..	0
September ..	22	0	28	4	3	15	1	19	2	2	..	0	..	0	..	0	..	0
Totals ..	222	0	150	17	9	85	8	75	7	6	26	0	0	0	58	1	0	0
Rate per cent.	11.3	6.0	..	9.4	7.1	..	9.3	8.0
Percentage of catch dissected.	35.7	..	24.1	13.5	..	12.1	4.2	0.3	..	0.2	..	0.4

G = Gut.

Gl = Salivary gland.

TABLE VIII.

Matkabadi village : house catch.

Month, 1935.	<i>A. culicifacies.</i> <i>A. fluviatilis.</i>		<i>A. varuna.</i>		<i>A. minimus.</i>		<i>A. aconitus.</i>		<i>A. jeyporiensis.</i>		<i>A. splendidus.</i>		<i>A. theobaldi.</i>		<i>A. jamesi.</i>		<i>A. pallidus.</i>	
	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+	No.	G+Gl+
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
October ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
November ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
December ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
January ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
February ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
March ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
April ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May (2) ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
June ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
July ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
August ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
September ..	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals ..	181	0	0	233	24	11	56	5	5	33	6	1	33	1	0	47	2	0
Rate per cent.	0	0	0	103	47	..	89	89	..	18.2	3.0	..	3.0	0	..	43	0	..
Percentage of catch dissected.	29.6	38.1	9.2	5.4	5.4	7.7

G = Gut.

Gl = Salivary gland.

TABLE IX.

Tikarapara and Monikol villages, houses and some cowsheds searched.

Month, 1935.	<i>A. culicifacies.</i>		<i>A. fluviatilis.</i>		<i>A. varuna.</i>		<i>A. minimus.</i>		<i>A. aconitus.</i>		<i>A. jeyporiensis.</i>		<i>A. splendidus.</i>		<i>A. maculatus.</i>			
	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +	No.	G+ Gl +		
October ..	9	0	4	0	0	2	0	0		
November		
December ..	30	0	10	0	0	2	1	0	1	0	0		
January ..	25	0	8	0	0	0	1	0	0	8	0	2	0	1		
February ..	41	0	5	0	0	..	0		
March ..	16	0		
April ..	85	0	2	0	0	1	0	0		
May ..	11	0		
June ..	212	0	4	1	0	4	0	0	1	0	0	1	0		
July ..	220	0	3	0	0	2	0	0	3	0	0	0		
August ..	213	0	17	1	1	6	0	0	5	2	0		
September		
Totals ..	862	0	48	2	1	17	1	0	11	2	0	13	0	2	0	0
Rate per cent.	4.1	2.1	..	5.9	0	..	18.2	0
Percentage of catch dissected.	90.3	..	5.0	1.8	1.2	1.3	..	0.2	..	0.2

G = Gut.

Gl = Salivary gland.

TABLE X.

Ambodala Dom village : catch in houses with cousheds inside them.

Month, 1935.	<i>A. culicifacies.</i>			<i>A. fluviatilis.</i>			<i>A. varuna.</i>			<i>A. minimus.</i>			<i>A. aconitus.</i>			<i>A. jeyporicensis.</i>			<i>A. splendidus.</i>			<i>A. maculatus.</i>			<i>A. theobaldi.</i>			<i>A. pallidus.</i>		
	<i>A. culicifacies.</i>			<i>A. fluviatilis.</i>			<i>A. varuna.</i>			<i>A. minimus.</i>			<i>A. aconitus.</i>			<i>A. jeyporicensis.</i>			<i>A. splendidus.</i>			<i>A. maculatus.</i>			<i>A. theobaldi.</i>			<i>A. pallidus.</i>		
	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+
October	100	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
November (2)	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
December	44	0	0	10	1	0	8	0	0	6	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
January	43	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
February	26	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
March (2)	63	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
April	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May	87	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
June	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
July	141	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
August	100	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
September *	233	0	0	3	0	0	2	0	0	0	0	0	0	0	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Totals	1,029	0	0	59	2	0	22	0	0	21	0	0	14	0	0	40	0	0	9	0	0	1	0	0	4	0	0	44	0	0
Rate per cent	3.4	0
Percentage of catch dissected.	82.8	47	1.8	1.7	1.1	3.2	0.7	0.1	0.3	..	3.6

G = Gut.

Gl = Salivary gland.

* September done in 1935 and 1936.

TABLE XI.

Lanjigarh Road : Bengal Timber Trading Company's coolie camp : house catch.

Month, 1935.	<i>A. culicifacies.</i>			<i>A. fluviatilis.</i>			<i>A. varuna.</i>			<i>A. minimus.</i>			<i>A. aconitus.</i>			<i>A. jeyporiensis.</i>			<i>A. splendida.</i>			<i>A. maculatus.</i>			<i>A. theobaldi.</i>			<i>A. pallidus.</i>								
	No.			G+Gl+			No.			G+Gl+			No.			G+Gl+			No.			G+Gl+			No.			G+Gl+			No.			G+Gl+		
	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+	No.	G+	Gl+			
October ..	64	0	0	10	1	2	1	0	0	1	0	0	2	0	0	1	0	0				
November (2) ..	56	0	0	17	1	0	6	0	0	1	0	0				
December ..	24	0	0	3	0	0	2	0	0				
January ..	21	0	0	14	0	0	2	0	0	1	0	0	1	0	0				
February ..	21	0	0	6	0	0				
March ..	3	0	0				
April ..	32	0	0				
May ..	49	0	0				
June *	31	0	0	1	0	0				
July ..	24	0	0	1	0	0	2	1	0				
August ..	170	1	0	5	0	0	1	0	0				
September ..	77	0	0	27	2	0	10	0	0	1	0	0	1	0	0				
Totals ..	572	1	0	83	4	2	22	1	0	3	0	0	1	0	0	3	0	0	1	0	0	5	0	2	0	0	2	0	0	0	0			
Rate per cent	0.2	0	..	48	2.4	..	4.5	0	..	0	0				
Percentage of catch dissected.	82.6	12.1	3.2	0.4	0.2	0.4	0.2	0.7	0.3				

G = Gut. GI = Salivary gland.

* Camp and Kakbhata combined.

TABLE XII.

Kakbhata village: catch in houses and cowsheds.

Month, 1935.	A. culicifacies.		A. fluviatilis.		A. varuna.		A. minimus.		A. jeyporiensis.		A. maculatus.		A. theobaldi.		A. pallidus.	
	No.	G+ Gl+	No.	G+ Gl+	No.	G+ Gl+	No.	G+ Gl+	No.	G+ Gl+	No.	G+ Gl+	No.	G+ Gl+	No.	G+ Gl+
October	56	0	1	0	1	0	1	0	5	0	2	0	2	0	1	0
November (2)	77	0	4	1	4	1	0	1	0	0	0	0	0	0	0	0
December	13	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0
January	24	0	2	1	1	0	0	0	1	0	0	0	0	0	0	0
February	12	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
March	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
April	32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
June *	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
July	196	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
August	24	0	0	1	8	1	0	0	0	0	0	0	0	0	0	0
September	55	0	2	1	9	0	1	0	1	0	0	0	1	0	0	0
Totals	495	0	135	10	6	25	2	3	0	1	7	0	0	0	2	0
Rate per cent	...	0	7.4	44	8.0	8.0	8.0	0	33.3
Percentage of catch dissected.	73.3	...	20.0	...	3.7	...	0.4	...	1.1	...	0.3	...	1.1	...	0.1	...

G = G⁺

Gl = Salivary gland.

* Catch counted in with Camp, Table XI.

From a study of Tables III to XII we arrive at the following totals:—

Species.	Number dissected.	Gut +.	Glands +.	Oöcyst rate.	Sporozoite rate.
<i>A. culicifacies</i> ..	4,744	3	0	0.06	0
<i>A. fluviatilis</i> ..	1,111	80	40	7.2	3.6
<i>A. varuna</i> ..	317	26	15	8.4	4.7
<i>A. minimus</i> ..	201	17	8	8.5	4.0
<i>A. aconitus</i> ..	107	1	0	0.9	0
<i>A. jeyporiensis</i> ..	318	4	0	1.3	0
<i>A. splendidus</i> ..	38	0	0	0	0
<i>A. maculatus</i> ..	13	0	0	0	0
<i>A. theobaldi</i> ..	19	0	0	0	0
<i>A. jamesi</i> ..	2	0	0	0	0
<i>A. pallidus</i> ..	68	0	0	0	0
<i>A. annularis</i> ..	6	0	0	0	0
TOTALS ..	6,944				

I think it can therefore be taken as amply proved that, in the parts of the Jeypore Hills covered by this investigation, only *A. fluviatilis*, *A. varuna* and *A. minimus* are concerned in the transmission of malaria. As the above figures indicate, their oöcyst and sporozoite rates show no significant differences. No differences in their breeding-place preferences have been discovered, and thus I think it is legitimate to treat them together as the *funestus* group, as was done earlier in this paper and in my previous publications. Their infectivity rates are very much higher than those found, for *A. minimus* only, in Assam (Lamprell, 1936), and are equal to those found, for *A. fluviatilis* only, by Measham and Chowdhury (1934) in the Anaimallai Hills. The gut infection in *A. aconitus* is, I think, the first to be discovered west of Burma (Covell, 1927).

A. jeyporiensis (type form) has not hitherto been found infected in nature*, Iyengar's positive specimens being the variety *candidiensis* (Iyengar, 1934 and 1934a). More dissections of both *A. aconitus* and *A. jeyporiensis* are required before these species can be completely acquitted of any part in the transmission of malaria in these hills. It is noteworthy, however, that three of the infections were encountered in January, when the infectivity index (*vide infra*) of the *funestus* group is rising to its second maximum, and *A. jeyporiensis* (*vide* Tables I and II) has just passed its numerical maximum, and is overflowing from cattle sheds into houses.

This is not the case, however, with *A. culicifacies*. In this case it would appear certain that the most widespread carrier of the Indian sub-region plays no part in the transmission of malaria in these hills, which are so notorious for the prevalence of the disease. A study of the individual village tables shows that dissections of this species in large numbers proved negative, whilst simultaneously high infection rates were recorded in the *funestus* group.

This startling discovery is not entirely contradicted by the results of earlier workers. Perry (1914) dissected a considerable number on the 3,000-foot plateau (*vide* Table B) without encountering a single infection. Stephens and Christophers (1902) refer to the presence of this species in large numbers at Salur, at the foot of the ghat leading to the 3,000-foot plateau, in

* Whilst this paper was passing through the press I find I overlooked the record of two gut infections in this species in Mysore [Nursing, Rao and Sweet (1934), *Rec. Mal. Surv. Ind.*, 4, pp. 243-251].

the presence of a nil spleen and parasite rate. They state (p. 36) 'At Salur, *A. culicifacies* (which elsewhere has been found infected by us) occurs in profusion, the breeding grounds being close at hand and extensive, yet there is no splenic enlargement and no malarial infection. The possibility of infection from a neighbouring area of high infection is a constant one, yet there is no malaria there. Further observations of similar instances are necessary before any explanation can be satisfactory'.

From the compilations of Covell (1927 and 1931) and subsequent investigations by Sweet and Rao (1931), Iyengar (1934), Lamprell (1936) and Mulligan and Baily (1936) gland infections have been found in *A. culicifacies* in the following localities :—

Ceylon—general.

Travancore.

Malabar.

Kanara.

Mysore.

Madras : Mopad*.

Nandyal*.

Penunkonda*.

Udayagiri*.

Ennur.

Central Provinces: (No locality given).

United Provinces: Saharanpur.

Kosi.

Punjab : Mian Mir.

Sind : Shikarpur.

Larkana.

Baluchistan : Quetta.

Assam : (No locality given).

Burma : Mazali.

This would appear to indicate that the species is a carrier throughout its Indian range, but actually there is a big territory, ranging from Lucknow to Assam, and south to Vizagapatam, from which either no dissections whatever are recorded or these have been negative or yielded gut infections only. Is there a big block of country in East Central India where the species, for some reason, is not acting as a carrier? I have found one gut infection at Bhadrak (Balasore district, Orissa), while out of 674 specimens dissected from the hyperendemic Singhbhum Hills only 4 infected guts have been found (in this area again the *funestus* group are obviously the principal carriers). Are we dealing with a real phenomenon [the 1,946 negative gland dissections by Timbres (1935) in North Bengal would strongly suggest this], or only with lack of information? It is obvious that much further and widespread work is necessary.

The obvious explanation as regards the Jeypore Hills that at once comes to mind is that *A. culicifacies* is much more attracted to cattle than to men. Tables I and II indicate at least a greater attraction to cattle sheds as a resting place, but there are plenty of the species resting in houses also. Only the results of precipitin tests, which it is hoped will shortly be commenced, can however decide whether *A. culicifacies* is almost entirely a cattle feeder in the Agency. But if so, why? Observational impressions would not lead one to think that there is any real difference between the numbers and stabling arrangements of cattle in these hills and on the plains generally. But the following interesting observations made by Mr. V. Venkat Rao, A.R.S.N.I., Malaria Inspector, East Coast Section of this railway, whilst on leave in Salur, do definitely indicate the superior attraction of cattle over men for the species.

* These localities are in the Eastern Ghats, of which the Jeypore Hills also form a part, but south of the Godavari valley gap.

'.....I went to Salur in February last, and I found immense breeding of *A. culicifacies* in the river and also in some fresh water wells. I know Salur from my childhood, but do not remember any epidemic of malaria having broken out there.....When I again went there in May, I took with me a catching tube, a hand lens, etc., to study the question for myself. I was putting up with.....an agriculturalist. Attached to his house is a cattle shed where two cows and four bulls are housed at night. For two days, I searched the dark rooms of the house for adult mosquitoes but found only one *culicifacies* ♀ and *C. fatigans* 3 ♂, 16 ♀ for the two days. When I made a search of the cattle shed, on the first day, I caught 60 *culicifacies* (the vast majority were females). The next morning I caught 64, and on the third day 58. As I did not know whether these mosquitoes were feeding on human beings at night and resting in the convenient cattle shed by day, I removed the cattle on the fourth day to another shed far away from the house, and then examined the shed for three days. My catches on those days were 3, 4 and 2 *culicifacies* respectively. On the evening of the last day the cattle were brought back to this shed, which was examined the next morning. The result of this examination was a catch of 78 *culicifacies*'.

(Mr. Venkat Rao later informed me that *A. culicifacies* did not increase in the house during the period when the cattle were removed.)

On writing to Sir Rickard Christophers about his observations of 1902, a reply was received to the effect that *A. culicifacies* were found like 'smoke' in the sheds. Sir Rickard goes on to say that the observations were simply made 'en passant', and apparently houses were not examined.

A second possible explanation is that *A. culicifacies* is not a single species, but, like *A. maculipennis* of Europe, is a mixture of two or more sub-species indistinguishable in the adult stage. This opens up a vista for an extensive investigation in systematic entomology. All that can be stated at present is that the maxillary index of *A. culicifacies* in the area covered by this paper is 13.3, which is within the human biting range, though much higher than the 12.0 of *A. minimus*, 12.1 of *A. varuna* and 12.3 of *A. fluviatilis*, found in specimens from these hills and those of Singhbhum*.

A third possible explanation is that in these hills *A. culicifacies* finds such suitable breeding facilities everywhere that it does not live long enough for the development of parasites to proceed to the sporozoite stage, as has been suggested for *A. subpictus* by Mehta (1934), though the argument is greatly weakened by the absence of even young oöcysts in *A. subpictus* (as that author points out), and in the present case by their great rarity in *A. culicifacies*. Also, in the present instance, the insect is obviously under unfavourable breeding conditions in the cold weather, and so infections might be expected in that season, which, as will be shown later, is the period of most active transmission of malaria by '*A. funestus*'.

The problem thus presented is not only fascinating, but obviously of extreme practical importance. The results already obtained will enable large economies to be effected in malaria control at the eight stations of the B.-N. Railway across the Jeypore Hills. Moreover, the application of this knowledge in conjunction with the herbage cover method described by Senior White

* A paper on the maxillary index of the Indian anophelines has been under preparation since 1933. Delay in publication is due to the difficulty of obtaining adequate numbers of caught specimens of some of the rarer species.

(1936) opens up the possibility of doing something for the control of rural malaria in the district generally. On this railway, control of *A. culicifacies* will be stopped forthwith at these eight stations. The results, as reflected in malaria cases, will be reported on in a subsequent part of this paper.

But the problem has a much wider aspect than this. Is there really a large section of East Central India where *A. culicifacies* is without importance as a carrier? The writer has similar work in progress in Singhbhum (Bihar), in the Satpuras (Central Provinces), and on the Orissa coastal plain, but there are vast areas beyond his jurisdiction that need the co-operation of other workers in regard to the points raised in this discussion.

Turning now to the results for individual villages tabulated in Tables III to XII, many points of interest emerge.

POLYPINDA VILLAGE was chosen because it was exposed to the results of intense breeding of *A. culicifacies* in the Kedapara River from January to April, at which period this river has forcibly reminded all who have seen both of the conditions in the Deduru Oya in Ceylon, where *A. culicifacies* breeding results in such intense malaria. The village is also within the influence of two hill nullahs breeding '*A. funestus*'. Its spleen rate (p. 54) is very high. Yet not a single infection has been found in a year's work, and the percentage of *A. fluviatilis* in the catch is excessively low. Why this should be so, will demand further investigations. The sectional malaria inspector having informed me that all his catches were made in large baskets, coated with cowdung, kept on the house verandas for storing tobacco leaves, and that adults were almost impossible to catch in the houses, I tested this statement and found it to be correct. The only possible explanatory fact noticed was that the houses swarmed with cockroaches (*Periplaneta* sp.) to an extent never seen elsewhere. Possibly these restless insects disturbed the resting mosquitoes, and whilst *A. culicifacies* could rest in the dimly-lit tobacco baskets, '*A. funestus*' with its preference for greater darkness could not do so, and therefore it repaired to the jungle by day.

CHATIKONA SUMMIT: This area is one of perennial irrigation, the majority of the paddy fields being cropped at least twice per annum. It is definitely the most intensely malarious station on the section, its protection involving the weekly oiling of some 12 miles of streams and drains and the paris green dusting of 239 acres of rice cultivation every fifth day. The village infectivity rates are expressive of the surrounding uncontrolled conditions. These are indicated by two examinations of Chatikona village (Doms), made in 1935. This village is well within the protection circle. Forty-two children were examined on each occasion.

				July (per cent).	December (per cent).	
Spleen rate	96	90.5	
Parasite rate	83	79	
Gametocyte rate	20	9.5	
The distribution of species being—						
<i>P. vivax</i>	15	(4 with gametocytes)		32	9 (1 with gametocytes)	24
<i>P. malariae</i>	6	(2 " ")		13	2 (No ")	5
<i>P. falciparum</i>	26	(4 " ")		55	27 (3 with ")	71

Multiple infections of all four possible combinations were found, and are counted separately in the species percentage.

This village is situated in the northern jaws of the pass, and it is believed that the nightly south wind referred to on p. 48 carries mosquitoes through the neck from immense breeding grounds below, to the village and the station, where its force spreads out and decreases. None-the-less, by combining daily 'fitting' of quarters and bi-weekly quinine with anti-larval measures the imported staff is maintained in good health, and the wives successfully bear and rear children.

The four villages from which dissections have been made are all north of the station. The results, as regards the *funestus* group, are combined in Table XIII. Infection is perennial, but if the monthly percentage of the total '*A. funestus*' catch [Table XIII, column (a)] is multiplied by the monthly gland infection percentage [Table XIII, column (b)], and the products divided by the lowest value obtained, an 'infectivity index' is obtained which is very instructive*, and refutes the *Gazetteer* statement on p. 48 that the rains are the most unhealthy period of the year. Unfortunately, this is seen actually to coincide with the cold weather, when the bulk of the touring of all Government officials is done.

In July and August it was noticed that there was an unexpected drop in the *funestus* group from Matkabadi village (Table VIII) which was not reflected in the catch at Bariguda village (Table VII). Now when the house catches for dissection were made, the inspectors were instructed to make catches in the cattle sheds of the villages for comparison, though not for dissection. The cattle shed catches for these two months did not show any special species changes to correspond with that in the houses.

Cattle shed catches.

Species.	BARIGUDA.		MATKABADI.	
	July.	August.	July.	August.
<i>A. culicifacies</i> ..	8	24	20	26
<i>A. fluviatilis</i> ..	0	1	0	1
<i>A. varuna</i> ..	1	1	1	1
<i>A. minimus</i> ..	8	1	1	6
<i>A. jeyporiensis</i> ..	0	1	2	0
<i>A. subpictus</i> ..	2	11	36	10
<i>A. vagus</i> ..	3	5	15	6
<i>A. pallidus</i> ..	2	0	2	0

On enquiring into the matter it was found that in these two months, at Matkabadi, the catches had been made, not in the Khond street as usual, but in the Dorr street, and the inspector pointed out that the latter had pig-sties attached to the houses, which the Khond street had not. This suggests that

*Owing to the fact that in November some additional catches were made, that one village had to be left unexamined in December, and that in July and August one village was searched in unsuitable houses (*vide infra*), the monthly values are not strictly accurate, but they serve as a relative measurement.

TABLE XIII.

Chatikona Summit : house catches in four villages for the funestus group.

Month, 1935.	<i>A. fluviatilis.</i>			<i>A. varuna.</i>			<i>A. minimus.</i>			Number of ' <i>A. funestus</i> ' in catch.	(a) Percentage of total ' <i>A. funestus</i> ' in catch.	Number of gland infections found.	(b) Percentage of infected glands.	(a) × (b).	Infectivity index.
	No.	G+	Gl +	No.	G+	Gl +	No.	G+	Gl +						
October ..	49	4	5	5	1	0	28	6	1	82	7.1	6	7.3	51.8	6
November ..	206	21	8	57	7	3	28	2	0	291	25.1	11	3.8	95.4	11
December ..	116	2	3	43	0	0	12	1	0	141	12.2	3	2.1	25.6	3
January ..	127	10	4	33	1	0	23	1	0	183	15.8	4	2.2	34.7	4
February ..	57	6	2	24	2	4	11	0	0	92	7.9	6	6.5	51.3	6
March ..	19	3	1	11	1	0	6	1	0	36	3.1	1	2.8	8.9	1
April ..	15	0	0	9	0	0	5	0	1	29	2.5	1	3.5	8.7	1
May ..	11	1	0	7	2	1	4	1	0	22	1.9	1	4.5	8.6	1
June ..	12	0	0	8	2	2	1	0	0	21	1.8	2	9.5	17.1	2
July ..	18	0	1	8	1	0	26	2.2	1	3.8	8.4	1
August ..	36	5	4	14	1	1	16	0	2	66	5.7	7	10.6	60.4	7
September ..	101	10	5	42	4	2	28	3	3	171	14.7	10	5.8	85.3	10
Totals ..	767	62	33	231	22	13	162	15	7	1,160	..	53	4.6
Rate per cent.	..	8.1	4.3	..	9.5	5.3	..	9.3	4.3

G = Gut.

Gl = Salivary gland.

'*A. funestus*' may prefer to rest in pig-sties, where available, and so explain the apparent absence of this group from certain villages, and the difficulty in catching them experienced in Ceylon by Carter and Jacocks (1929), pp. 73-74. In Sinhalese villages pigs are common. The Indian domestic pig is an unlovable creature, and its sty worse. A special sty is being built in a village to investigate this point. But, even if sties are a resting place, the pig would not appear to be able to divert '*A. funestus*' from man, judging from spleen rates.

The villages of TIKARAPARA, MONIKOL and AMBODALA (Tables IX and X) exhibit in lesser degree the phenomenon noted at Polypinda (Table III), of a preponderating uninfected *A. culicifacies* population, and of a very low infectivity rate in the *funestus* group, quite insufficient to account for the spleen rates. After the last monthly catch, this was thoroughly investigated in the third of these villages. The result was most instructive, indicating the pitfalls that lie in wait for the unwary. Being a Dom village, many of the houses have cattle sheds combined. One house was studied in detail. Entering by the front door we found two living rooms, one entirely unlighted. Behind this was a back veranda, lit by two overlapping eaves. Behind this was what was really a separate building, kitchen on one side, cattle shed on the other, separated by a half-wall, about three feet high. A back door communicated with a lane. The catch was instructive: in the front half of the house *A. fluviatilis* 3 ♀, *A. varana* 2 ♀, *A. minimus* 1 ♀, *A. jeyporiensis* 1 ♀, were caught: in the back half, *A. culicifacies* 5 ♀, *A. subpictus* 2 ♀, all of which were taken in the cattle room, except one *A. culicifacies*, which was collected in the kitchen. The cattle were out at pasture during the examination. The inspector had each month searched the more easily examinable back half of the house, which did not involve disturbing the women, thus missing the '*A. funestus*' that really were in the living portions of the houses almost entirely. We were informed that people slept in the kitchen as well as in the front rooms. A second year's work on these villages has been started, the results of which will appear in a subsequent part of this paper.

THE TWO VILLAGES AT LANJIGARHI ROAD (Tables XI and XII) are instructive. This station is situated in forest country, with, however, considerable paddy in stream beds artificially widened. There are only three small hamlets of a few houses each, on the periphery, or within a mile or more from it. Comparing the two tables, we see one of these, Kakbhata, with infective '*A. funestus*' present from September to February, whereas in the coolie camp at the centre of protection, oöcyst rates are much lower and sporozoites have only been found once, in October. The explanation possibly is that, with such a small human blood supply available in the surrounding area, the large blood supply available in the camp causes flight from considerably beyond the normal half-mile radius that is protected. None-the-less, as all water suitable for '*A. funestus*' breeding is treated, an anopheline, which has acquired infection in the camp, would be forced to make a very long journey or journeys before ovipositing, and would seldom survive to return with sporozoites in its glands.

Tables XII and XIII, moreover, supply the answer to the original object of the investigation. Where irrigation is not perennial, the period March to July is one of no transmission. Forest areas must always experience higher humidity than dry paddy lands, such as surround the villages in British

territory at that season, and, at Lanjigarh Road, moreover, the perennial Sundel River flows along the periphery of the protected zone to the west. If such conditions do not permit of transmission, then all the control measures except those at Chatikona Summit can be closed down in these months. This hypothesis, also, will be put to practical proof in the coming year, and the results reported later.

SUMMARY AND CONCLUSIONS.

1. At 1,000 feet level in the Jeypore Hills, 6,944 dissections of twelve species of *Anopheles* have been made in the year. Six species have been found infected, but only three, the closely related *A. fluviatilis*, *A. varuna* and *A. minimus*, have been found infective.

2. The infected and infective rates in these three species are so similar that for practical purposes they can be treated as one, the '*funestus*' group, it being well known that no differences in their respective breeding places have been discovered.

3. A number of 4,744 *A. culicifacies* has been dissected, with the discovery of three infected guts only (0.06 per cent). This species, which undoubtedly dominates the anopheline fauna, plays no part in the transmission of malaria in these hills. Some evidence is quoted to indicate that possibly this species is not of pathogenic importance over a wide tract of East Central India.

4. Where perennial irrigation is available, infection is perennial. In other localities there is no transmission between February and August.

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A PRELIMINARY NOTE ON THE ACTION OF PLASMOQUINE IN PREGNANCY.

BY

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*(Inquiry under the Indian Research Fund Association, at the Haffkine
Institute, Bombay.)*

[23rd November, 1936.]

THE ecobolic action of quinine has led to its very cautious use in the treatment of malaria during pregnancy. The physician's ideas about quinine causing abortion have, however, gradually changed during recent years and modern pharmacologists also have expressed the view that the illness is more likely to cause abortion than quinine. Thus, Clark (1932) stated that moderate doses of quinine could be given to pregnant women in malarious districts and Micks (1935) observed that when abortion occurred during an attack of malaria it was probably the effect of the illness and not of quinine.

Introduction of synthetic antimalarials has brought the question of treatment of malaria during pregnancy again into prominence. The clinical and experimental observations regarding the use of plasmoquine in pregnancy were summarised by Manson-Bahr in 1932 and the same author recorded his own clinical observations that a combination of plasmoquine and quinine was well tolerated by pregnant women. Chopra and his co-workers (1933) studied the action of plasmoquine on the uteri of cats and rabbits, and concluded that the lowest effective concentration which would stimulate the isolated uteri of these animals was 1 in 200,000, while a dose as high as 2 mg. per kg. given intravenously caused just a slight increase in the tone of the uterus *in situ*. Epstein (1933) thought that low concentrations like 1 in 500,000 caused a contraction of the isolated cat's, rabbit's and guinea-pig's uterus, while concentrations greater than 1 in 100,000 produced relaxation of the tone. He further observed that doses of 1 mg. per kg. caused a contraction of the intact uterus, and in some experiments this effect was produced by smaller doses which did not cause a fall in blood pressure. Both these observers however agreed that plasmoquine was comparatively safe in pregnancy.

In the present investigation, experiments on the animal uteri were repeated and some more experiments were made to study the effect of plasmoquine on the course of pregnancy.

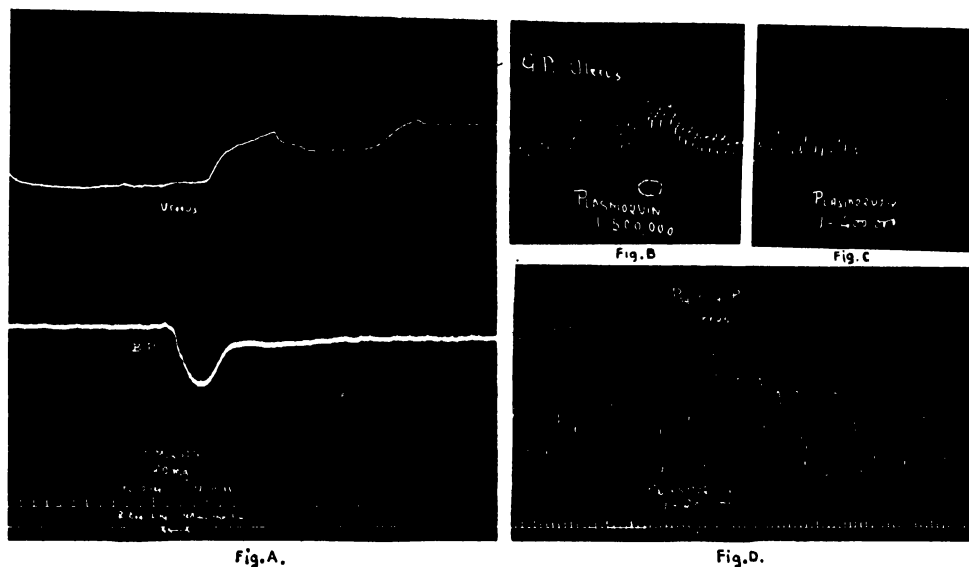
In all the experiments given below, plasmoquine was used in the form of solution supplied by the manufacturers in 3 c.c. ampoules.

ACTION OF PLASMOQUINE ON THE UTERUS OF EXPERIMENTAL ANIMALS.

The action of plasmoquine on the uteri of cats, dogs, rabbits and guinea-pigs was studied *in vitro* and *in vivo* experiments.

Experiments on the isolated uterus were conducted in the usual way by suspending it in Locke's solution in a Dale's bath for isolated organs. The temperature was kept constant at 38°C. and air bubbled through continuously to oxygenate the solution. Different quantities of plasmoquine were added to the bath so as to give concentrations of the drug varying from 1 in 1,000,000 to 1 in 50,000. The uteri of cats, rabbits and guinea-pigs were found to be more susceptible to the action of plasmoquine than those of dogs.

GRAPH 1.



- Fig. A.—♀ Dog, 12 kg., urethane morphine anaesthesia. Tracings from above downwards:—Uterus = Uterine movements; B. P. = Carotid blood pressure; T. = Time 10 seconds interval; Base line. Plasmoquine 20 mg. given intravenously at the mark in base line. Note the contraction of the uterus and fall in blood pressure.
- Fig. B.—Isolated non-pregnant guinea-pig's uterus. Note plasmoquine 1 in 500,000 produces a slight increase in the tone.
- Fig. C.—Same as Fig. B. Note the effect of 1 in 400,000 concentration of plasmoquine.
- Fig. D.—Isolated pregnant guinea-pig's uterus. Note plasmoquine 1 in 200,000 inhibits the uterine contractions.

The effect of plasmoquine on the isolated uteri of guinea-pigs, both pregnant and non-pregnant, is shown in Graph 1. It will be seen that a non-pregnant uterus responded to a 1 in 500,000 concentration of plasmoquine by a slight contraction (Graph 1, fig. B) and to a 1 in 400,000 by a contraction

and inhibition of automatic movements (Graph 1, fig. C). The effect of a 1 in 500,000 concentration of plasmoquine on the rabbit's uterus is shown in Graph 3, fig. A. These effects in such dilutions are, however, not very common and usually a 1 in 300,000 concentration of plasmoquine is required to produce a slight contraction of the isolated uterus. Higher concentrations of plasmoquine such as 1 in 200,000 to 1 in 100,000 produce the opposite effect, viz., a relaxation of tone and inhibition of automatic movements.

The action on the pregnant uterus is of the same nature, lower concentrations producing a contraction and higher a relaxation of the isolated pregnant uterus. Graph 1, fig. D, shows the effect of a 1 in 200,000 concentration of plasmoquine on the movements of a pregnant guinea-pig's uterus. It will be seen that even such a high dilution produced inhibition of the uterus.

In intact animals, the uteri of cats and rabbits were found to be more susceptible to the action of plasmoquine than those of dogs. In cats a dose of approximately 1 mg. per kg. usually produces a slight contraction of the uterus while in dogs a dose of about 1.5 to 2 mg. per kg. is required to produce the same effect. Graph 1, fig. A, shows the result obtained in one such experiment. The non-pregnant dog under morphine-urethane anaesthesia was given about 1.6 mg. per kg. of plasmoquine intravenously. This injection produced a moderate contraction of the uterus and a fall in blood pressure. That the contraction was due to the action of the drug and not to vasomotor depression was shown by the fact that when a similar fall in blood pressure was produced by bleeding the animal it did not affect the movements of the uterus in any way.

Pregnant uteri of cats and dogs responded to an intravenous injection of plasmoquine in the same way as did the non-pregnant ones.

ACTION ON THE HUMAN UTERUS.

The action of plasmoquine on the isolated human uterine muscle strip was investigated by suspending the strip in Locke's solution in the same way as in the case of animal uteri. The temperature was maintained constant at 37.5°C. and oxygen was bubbled continuously through the solution. Plasmoquine was added to the bath in different quantities to give the desired concentrations. The results obtained in one such experiment are shown in Graph 2. In this experiment the uterine muscle was obtained from a patient 3½ months' pregnant. A small narrow strip was removed at the time of an operation which was performed for therapeutic reasons. The strip, immediately on removal, was transferred to a bottle containing Locke's solution, put on ice and taken to the experimental room. It was suspended in oxygenated Locke's solution immediately afterwards and showed the normal uterine contractions within 15 minutes. Different quantities of plasmoquine were added to give final concentrations varying from 1 in 1,000,000 to 1 in 75,000. Graph 2, fig. A, shows the effect of a 1 in 1,000,000 concentration of plasmoquine. A very slight increase in the tone was produced by this concentration. There was a distinct increase in the tone of the muscle after a 1 in 500,000 concentration of plasmoquine (Graph 2, fig. B). Higher concentrations such as 1 in 250,000, however, produced a slight relaxation of the tone (Graph 2, fig. D), while still higher concentrations such as 1 in 75,000 produced a definite relaxation of the tone and inhibition of automatic movements (Graph 2, fig. C).

Qualitatively similar results were obtained in the case of a non-pregnant human uterus, higher concentrations producing a relaxation and lower, a

GRAPH 2.

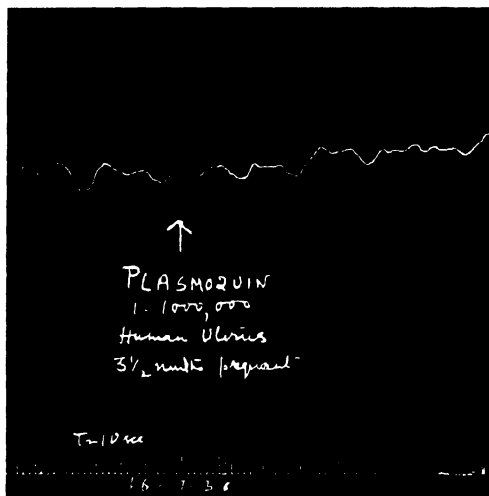


FIG. A.

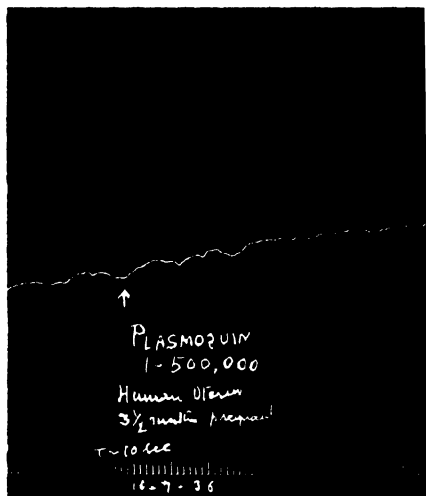


FIG. B.

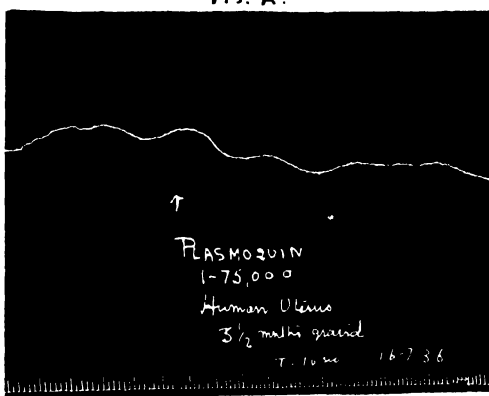


FIG. C.

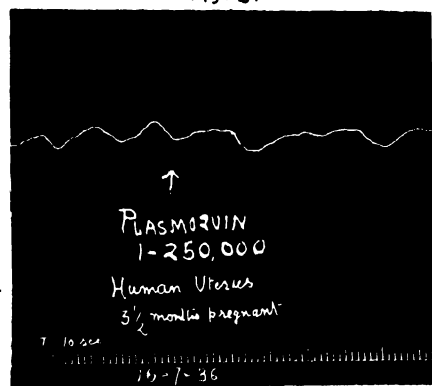


FIG. D.

Fig. A.—Isolated human uterine muscle strip. The patient from whom this strip was removed was 3½ months' pregnant. Note plasmoquine 1 in 1,000,000 produces a very slight contraction of the uterine muscle.

Fig. B.—Same as Fig. A. Shows the effect of 1 in 500,000 concentration of plasmoquine. Note the slight increase in the tone of the uterine muscle.

Fig. C.—Same as Fig. A. Note the relaxation of tone and inhibition of automatic movements produced by a 1 in 75,000 concentration of plasmoquine.

Fig. D.—Same as Fig. A. Note the slight relaxation of the tone of the uterine muscle by a 1 in 250,000 concentration of plasmoquine.

contraction of the uterine strip. It was noted however that the pregnant uterus was more sensitive to the action of plasmoquine than the non-pregnant one.

EFFECTS OF PLASMOQUINE ON THE ACTION OF OTHER UTERINE STIMULANTS.

Frank as early as 1930 observed that plasmoquine was able to antagonise the stimulant effect of quinine on the uterus. Epstein (*loc. cit.*) came to the conclusion that ordinary doses of plasmoquine did not modify the action of quinine on the uterus. This point was investigated further, and experiments were made to see the action of plasmoquine on known uterine stimulants such as quinine and pituitrin (Parke, Davis & Co.).

GRAPH 3.

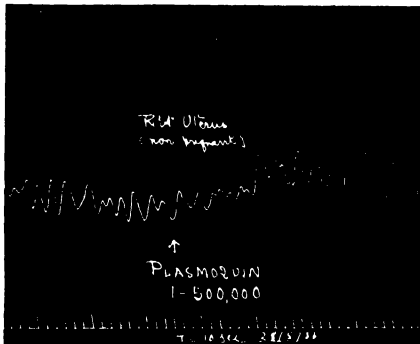


Fig. A.

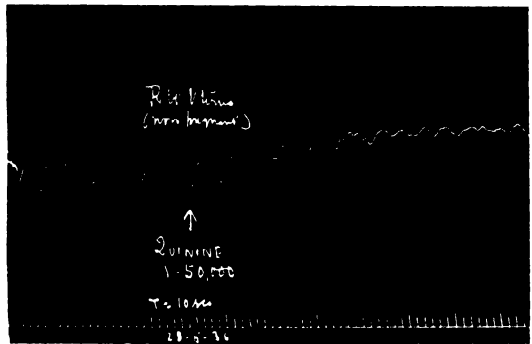


Fig. B.

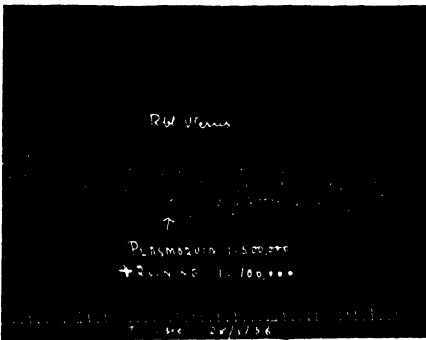


Fig. C.

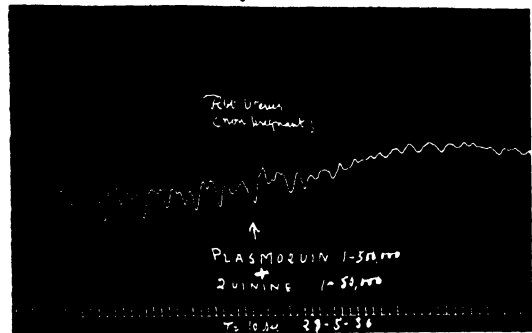


Fig. D.

Fig. A.—Isolated rabbit's uterus, non-pregnant. Note the slight contraction produced by a 1 in 500,000 concentration of plasmoquine.

Fig. B.—Same as Fig. A. Shows the effect of 1 in 50,000 concentration of quinine.

Fig. C.—Same as Fig. A. Shows the effect of a combination of 1 in 500,000 plasmoquine and 1 in 100,000 quinine. Note that there is practically no response.

Fig. D.—Same as Fig. A. Note that a combination of 1 in 500,000 plasmoquine and 1 in 50,000 quinine produces a contraction of the uterus which is in no way greater than that produced by quinine alone.

The effect of plasmoquine on the stimulant action of quinine on the isolated rabbit's uterus is shown in Graph 3. Fig. A shows that the uterine muscle responded to a 1 in 500,000 concentration of plasmoquine by a slight

contraction and a 1 in 50,000 concentration of quinine by a moderate contraction (Graph 3, fig. B). A combination of plasmoquine 1 in 500,000 along with quinine 1 in 100,000 however failed to elicit any appreciable response (Graph 3, fig. C) and plasmoquine 1 in 500,000 with quinine 1 in 50,000 produced a contraction of the uterine muscle which was in no way greater than that produced by 1 in 50,000 quinine alone (Graph 3, fig. D). It appears therefore that on the isolated uterus not only does plasmoquine antagonise the action of quinine but that, when given in smaller doses along with quinine, it loses its own stimulant action on the uterine muscle.

Similarly a previous application of plasmoquine in low concentrations (1 in 300,000) lessens the stimulant action of pituitrin on the isolated uteri of rabbits and guinea-pigs. With higher concentrations (1 in 100,000 and more) this effect is even more marked. On the isolated uterus, therefore, plasmoquine produces an effect which prevents the uterine muscle from responding to quinine and pituitrin normally.

In the intact animal, however, larger doses of plasmoquine are required to produce the same result. In anaesthetised cats, the contraction of the uterus produced by a small dose of quinine or pituitrin is of the same strength before as after 1 mg. of plasmoquine per kg. of body weight given intravenously. Repeated small doses (1 mg. per kg.) of plasmoquine however showed a diminished response to pituitrin when compared with the contraction of the uterus produced by the same dose of pituitrin before administration of plasmoquine. The results were therefore in agreement with those of Epstein (*loc. cit.*).

EFFECT OF TOXIC DOSES OF PLASMOQUINE ON THE COURSE OF PREGNANCY.

Toxic doses were administered to pregnant guinea-pigs orally or hypodermically. Six guinea-pigs received a dose of about 20 mg. per kg. of body weight. In four of these the pregnancy was well advanced and the animals would probably have delivered a litter in the course of the next three or four days. In the other two guinea-pigs, pregnancy was fairly advanced. In all these cases the toxic dose of 20 mg. per kg. produced death of the mother and foetus without producing any premature delivery. In four other guinea-pigs with advanced pregnancy a total dose of 15 mg. per kg. of body weight was given in divided doses over a period of three days. All the guinea-pigs died within one week of the beginning of the injections, without showing premature delivery. Another guinea-pig (weight 800 gm.) which was well advanced in pregnancy received two doses of 5 mg. and 4 mg. on the first and third day respectively, hypodermically. The animal died on the sixth day without showing any sign of premature delivery. In one case, however, a toxic dose of plasmoquine produced premature delivery. A guinea-pig weighing about 850 gm. was given doses of 2, 3 and 4 mg. of plasmoquine hypodermically on the first, third and seventh day, thus making a total of 9 mg. in seven days. The animal delivered a litter of three on the eighth day, all of which were born prematurely with membranes intact. The mother died on the tenth day.

It will be seen therefore that single large doses of plasmoquine produce death of the mother along with the foetus without producing premature delivery irrespective of the stage of pregnancy. The action of repeated toxic doses also

appears to be the same. There appears to be a general agreement about the relative harmlessness of therapeutic doses of plasmoquine from clinical studies. This point also requires experimental elucidation and experiments are already in progress in this institute to determine the effect of therapeutic doses of plasmoquine on the course of pregnancy.

DISCUSSION.

Experiments on the animal and human uteri show that *in vitro* the human uterus is as sensitive to the action of plasmoquine as the animal uteri. It is likely therefore that *in vivo* the human uterus responds in the same way as do the animal uteri. In all the *in vivo* experiments where the action of plasmoquine on the animal uteri was recorded graphically, the animal was under some form of anæsthesia, and this procedure may modify the true action of the drug. Administration of toxic doses of plasmoquine to pregnant guinea-pigs, however, show that, even without anæsthesia, plasmoquine does not tend to increase uterine contractions so as to induce abortion. A dose of 20 mg. per kg. of body weight is a very toxic dose, and an equivalent toxic dose of quinine is very likely to produce abortion. The experiments, therefore, indicate that plasmoquine is less likely to produce abortion than quinine. In order to know the action of therapeutic doses of plasmoquine, however, experiments will have to be made on animals with doses equivalent to the therapeutic doses in man. Such experiments are already in progress and will be reported later.

Experiments on the isolated uteri indicate that a combination of plasmoquine and quinine mutually antagonises the stimulant effect of each on the isolated organs. Whether this holds true for the intact animal is doubtful. There is no indication, however, to show that such a combination enhances the ecboic action of either. As a combination of the two drugs has been found clinically to be very useful in the treatment of malaria, such a combination may be recommended for use in pregnancy.

SUMMARY AND CONCLUSIONS.

(1) Plasmoquine contracts the isolated uteri of cats, dogs, guinea-pigs and rabbits in low concentrations (1 in 500,000 to 1 in 300,000) and relaxes them in higher ones (1 in 100,000 and more).

(2) The human isolated uterus responds in the same way as the animal uterus.

(3) Small doses (1 to 2 mg. per kg.) of plasmoquine given intravenously contract the uterine muscle slightly in intact animals.

(4) Low concentrations of plasmoquine and quinine mutually antagonise the stimulant effect of each on the isolated uterus.

(5) Toxic doses of plasmoquine do not produce abortion in pregnant guinea-pigs even though pregnancy is well advanced.

ACKNOWLEDGMENTS.

My thanks are due to the Director, Haffkine Institute, Bombay, for providing funds, facilities and assistance to carry out these experiments. I am also indebted to Dr. Urehs and Dr. Soltner of the Haverro Trading Co. for supplying me with sufficient quantities of plasmoquine for my experiments.

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SOME PROBLEMS IN THE HOST-PARASITE RELATIONSHIP IN THE MALARIAL INFECTIONS OF MAN AND OTHER ANIMALS*.

BY

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(Late Director, Malaria Survey of India).

[1st December, 1936.]

THE problems associated with the relationship of the malarial parasite to its vertebrate host are innumerable, and very many are the lacunæ in our knowledge of this subject. It is impossible in the time at my disposal to do more than touch on the fringe of the many aspects that it presents. An attempt will be made, however, to give a brief summary of the investigations carried out in the laboratories of the Malaria Survey of India, and to indicate some of the difficulties encountered, as well as lines for future research that the results suggest.

Our work has been mainly connected with the experimental infection of *Silenus (Macacus) rhesus* with *Plasmodium knowlesi* and *P. cynomolgi*, natural parasites of the Malayan monkey, *S. (M.) irus*.

The fact that these infections have been studied in a primate host should make the results and deductions less liable to criticism, when compared with human malarial infections, than has been the case with the avian disease. Many of the findings have helped to confirm and extend observations on human malaria, and to shed new light on its epidemiology and treatment.

In any study of the reaction of the vertebrate host to the effects of infection with Plasmodia, one must consider, especially, the following factors :—

- (A) The species and strain of parasite responsible for the infection, and
- (B) the reaction of the host infected by the parasite.

* This paper was read to open the discussion upon 'The Biology of the Malarial Parasites of Man and Animals' in the Section on Medical, Veterinary and Agricultural Zoology and Parasitology at the Second International Congress of Microbiology (1936).

(A) THE SPECIES AND STRAIN OF *PLASMODIUM* RESPONSIBLE FOR THE INFECTION.

(1) DETERMINATION OF THE PRESENCE AND TYPE OF INFECTION.

At present there is only one certain method of diagnosing the occurrence of a malarial infection, and that is by the finding of parasites. Various serological and other methods have been reported, but none of these has so far proved satisfactory in actual practice. The technique devised by Sinton and Mulligan (1932), for isolating large amounts of parasite substance from infected monkeys, opens up many lines for further research into better diagnostic methods and also into the possibility of artificial immunisation.

Although the cultivation of the malarial parasites *in vitro* held out great hopes of usefulness in the diagnosis of malarial infections, yet little or no progress has been made along these lines since Bass (1911) originally described his method a quarter of a century ago.

Apart from the diagnosis of infection, there is as yet no *certain* means of determining when an infection has been *permanently* eradicated, a matter of much importance in immunological studies. While in animals, it is possible by a combination of methods to arrive at a tentative conclusion on this point, such experiments would not always be advisable with the human subject (Sinton and Mulligan, 1933a).

After an infection has been detected, the accurate specific identification of the parasite responsible for it is one of the first essentials in any research work.

The study of avian malarial parasites by Hartman (1927) and others has helped greatly by showing that, in the past, a large number of species of bird Plasmodia were included under the common term of 'Proteosoma'*

Sinton and Mulligan (1932a; 1933) have attempted to clear up some of the confusion which existed in the diagnosis and nomenclature of the Plasmodia of the lower monkeys of the Old World. They have shown that the Oriental monkey, *S. irus*, is naturally infected with, at least, three different species of *Plasmodium*, namely *P. knowlesi*, having a 24-hour cycle of schizogony, *P. cynomolgi*, which has a 48-hour cycle, and *P. inui* with a 72-hour cycle (Sinton and Mulligan, 1933a; Sinton, 1934; Mulligan, 1935). As *S. rhesus*, the common brown monkey of northern India, can be infected easily with these three species by blood inoculation, this has opened up a wide field for experimental research into the malaria of the primates.

Although 50 years have elapsed since Laveran discovered the malarial parasite, until recent years only the three classical species were universally recognised. We have now an acknowledged fourth—*P. ovale*. There is, however, much evidence to support the view that, under the term 'malignant tertian' or 'subtertian' parasite, two or more distinct species of *Plasmodium* are included. Apart from its scientific interest, the fact that different species of Plasmodia have been found to react differently to different drugs, makes it important that a more exhaustive study of the species of human malarial parasites should be undertaken. Such a study might also help to shed fresh

* Many references to this work have been given by Mulligan and Sinton (1933).

light upon the ætiology of blackwater fever and the suggestion that *P. tenue* may have a definite relationship to this syndrome.

It must be remembered, in making any intensive study of a species of *Plasmodium*, that mixed infections are not very uncommon in nature. This has given rise to much confusion in many of the older descriptions of these parasites (Sinton and Mulligan, 1933a).

(2) DIFFERENT RACES OR STRAINS OF THE SAME SPECIES OF *PLASMODIUM*.

Recent work with experimental infections of human and simian parasites has shown that one morphological species of *Plasmodium* may be made up of several different races or strains. These strains have been found to differ not only in their immunological properties but, in some instances, in their effects on the host and also in their reaction to drugs. This discovery is of the greatest practical importance, and has already helped to throw much light on several problems in the epidemiology and treatment of malaria.

(a) IMMUNOLOGICAL DIFFERENCES IN STRAINS OF *PLASMODIA*.

The work on therapeutic malaria in Europe and America has shown that a person chronically infected with one strain of *P. vivax* develops a considerable degree of tolerance to the infection, and also to superinfection with the same strain. On the other hand, the results of superinfection with a different strain of the same morphological species suggest that, while a certain amount of common 'species' tolerance has been developed as a result of the original infection, yet this is insufficient to counteract completely the clinical effects of the heterologous superinfection. In some instances, the clinical manifestations of the latter infection may be almost, if not quite, as severe as those of the primary one*.

In avian malaria, however, the evidence available suggests that the tolerance developed to different species and strains of *Plasmodia* is not always so clearly specific in character, and that a considerably greater degree of cross-immunisation exists*.

Superinfections, both homologous and heterologous, have been carried out in our laboratories with 7 different strains of *P. knowlesi*. The animal used was *S. (M.) rhesus*, which shows a very high degree of susceptibility to this parasite. The following findings confirm and extend the results recorded in human malaria :—

(i) A chronic or latent infection with one strain of *P. knowlesi* (or of *P. cynomolgi*) confers a considerable degree of tolerance to superinfection with the same strain of parasite.

(ii) Single infections or multiple homologous superinfections with one strain do not produce any high degree of tolerance against the clinical manifestations of superinfections with a different strain of the same species of *Plasmodium*, although they may modify the effects slightly.

These findings show that, in each morphological species of *Plasmodium*, there may be contained various races or strains having different 'antigenic' elements. This has an important bearing upon the acquisition of individual

* Much of this work has been summarised by Mulligan and Sinton (1933).

and mass tolerance by the inhabitants of malarious countries, and upon the question of 'salted labour' (Sinton and Harbhagwan, 1935; Sinton, 1935).

(b) DIFFERENCES IN PATHOGENICITY OF STRAINS OF PLASMODIA.

Differences in the pathogenic effects of what were apparently the same species of *Plasmodium* have been reported from different parts of the world. It was uncertain, however, to what extent these variations were due to differences in the susceptibility of the hosts infected. Recent work on therapeutic malaria has shown that these variations in pathogenicity actually exist in different strains of human Plasmodia. Thus, the indigenous Dutch strain of *P. vivax* is reported to produce much milder effects than the Madagascar one, and the Italian strains of *P. falciparum* than the Indian ones. Similar variations have been found in the intensity of infections produced by different strains of *P. knowlesi* in *S. rhesus*.

(c) VARIATIONS IN THE REACTION OF DIFFERENT STRAINS TO THERAPEUTIC AGENTS.

The work on experimental malaria in man has shown that different strains of the same species of *Plasmodium* may respond differently to the curative action of the same therapeutic agent. Thus, the workers in Holland report that neosalvarsan has a powerful action on the severe Madagascar strain of *P. vivax*, and but a weak one on the indigenous Dutch strain of the same parasite. On the other hand, with ordinary quinine treatment only about 50 per cent of the infections with the latter strain relapse, as compared with about 100 per cent with the former infection. Sinton (1930), from a study of several thousand infections with *P. vivax* in North India, found that certain infections were extremely difficult to cure radically with the cinchona alkaloids, but that such refractory infections reacted very favourably to treatment with quinine and plasmoquine. James (1932) reports that infections with the Rome strain of *P. falciparum* needed ten times as much quinine to control them as did infection with the Indian strain of this species. Infection with the latter strain was comparatively easily cured permanently with quinine, and the former reacted well to atebirin therapy.

These variations in the effects of different drugs, on what were apparently the same morphological species of *Plasmodium*, were so marked that James (1932) states that 'the remarkable contrast brought out by therapeutic tests raises the question whether, from the chemo-therapeutic point of view, the biological susceptibility of a parasite to the action of drugs may not be more important than its morphological characters—strains may be more important than species'.

(3) VARIATIONS IN THE PATHOGENICITY OF DIFFERENT SPECIES AND STRAINS OF PLASMODIUM.

That infections with different species of *Plasmodium* vary markedly in the severity of their manifestations has been known for many years. As pointed out above, it has recently been found that strains of the individual species also vary in their pathogenicity in the vertebrate host. Some workers believe that the degree of 'virulence' of a strain is not a fixed character, but may vary under conditions such as (a) by passage through a very susceptible

vertebrate or insect host, (b) by passage directly from the insect as compared with blood inoculation, and (c) by an increased dosage of infection.

Before one can discuss any increased 'pathogenicity', 'toxicity', or 'virulence' of a strain of parasite, one must consider the factors which may be responsible for such effects. While one speaks in general terms of the 'toxins' of the malaria parasite, there is as yet no precise knowledge of the nature of these hypothetical substances. It is still unknown exactly how the pathogenic effects of the parasites are produced. The most widely accepted theory is that it is of the nature of 'protein shock', but there is no certain indication of the exact character of the foreign substance responsible for such shock. Now that it is possible to isolate large amounts of parasite substance from monkey infections, many new lines are opened up for the investigation of this subject.

The terms 'virulence' and 'toxicity' are often seen in the literature of malaria and are usually employed in the wider sense of 'pathogenicity' or 'power to produce pathogenic effects'. *Apart from the question of host susceptibility*, the degree of pathogenicity of a parasite depends on two distinct conditions, which should be differentiated clearly in any work on the subject :—

(a) The power of the parasite, by reason of its physical vigour and vitality, to establish itself and multiply freely in the host, irrespective of the individual toxicity of each parasite. Under these conditions, the extent of the pathogenic effects may depend upon the 'intensiveness' of the parasitic infection.

(b) The amount of 'toxin' which each individual parasite can produce. This may be called the 'toxicity' of the individual parasite.

These two factors taken together determine the 'virulence', 'pathogenicity', or 'aggressivity' of the parasite, in relation to the susceptibility of the host. Thus a severe attack of the disease might be caused by a high prevalence of parasites of low toxicity, or by a few parasites of high individual toxicity. It is also probable that a large output of 'toxins' would tend to lower the natural defences of the host, and so encourage a greater proliferation of the parasites. A careful study of the 'pyrogenic threshold', by exact enumerative methods in a large number of cases, would probably help to show which of these two factors was the chief element in any recorded differences in 'virulence'.

(B) THE REACTION OF THE HOST TO INFECTION WITH PLASMODIA.

The pathogenic effects produced by a parasite vary inversely as the resistance of the host to the multiplication of the parasites and to the effects of its 'toxins'. Such resistance may be either (1) a natural characteristic of the host, or (2) an acquired condition.

(1) NATURAL RESISTANCE OR TOLERANCE TO INFECTION.

It was suspected for many years that individuals vary very considerably in their resistance to, or tolerance of, infection by Plasmodia. The work on induced malaria has shown that, apart from any question of previous infection,

normal individuals do not show a uniform susceptibility to infection with these parasites. Some persons exhibit such a high degree of resistance or tolerance that few or no clinical manifestations are detectable, even if infection actually develops. Other individuals are very highly susceptible and exhibit exceptionally severe attacks of the disease, while a third group is intermediate between these two and may be said to react normally to infection.

In highly endemic malarial areas, a large proportion of the inhabitants appears to possess a high degree of natural tolerance to the pathogenic action of local strains of *Plasmodium*. This suggests that the less tolerant persons are killed off by the disease during childhood, while the more resistant survive to adult life and propagate their species. Such a condition of resistance might possibly be transmitted as a dominant Mendelian character, and, by a weeding out of the susceptibles during childhood, races have been evolved in whom the majority possess a high degree of resistance to the pathogenic effects of infection with the local strains of parasite (Sinton, 1935). The natural tolerance of *S. irus* to *P. knowlesi* makes this monkey a suitable subject for the study of this problem.

The occurrence of such a natural tolerance in a population has a very distinct practical application in relation to mass treatment and to labour problems. This has been discussed by Sinton and Harbhagwan (1935) and by Sinton (1935a).

(2) ACQUIRED TOLERANCE TO MALARIAL INFECTIONS.

As mentioned previously, it has been found that a host suffering from a chronic infection with one strain of either *P. vivax* or *P. knowlesi* develops a high degree of tolerance to the acute clinical manifestations of infection with the same strain of parasite. Our present information suggests that the degree and duration of this tolerance may be enhanced by multiple homologous superinfections, but the reason for such an increase requires further study (Mulligan and Sinton, 1933a).

Such homologous tolerance due to chronic infection with one strain of parasite is not highly efficacious against superinfection with a heterologous strain of the same species, although there is evidence suggesting that some strains possess certain 'antigenic' elements in common (Sinton and Harbhagwan, 1935). There also appears to be some degree of common 'species' tolerance developed between infections with parasites of the same species. This is shown by the fact that the clinical manifestations of heterologous superinfections are usually less severe than are primary infections. It is suggested that this may be due either to (i) some common 'species' antigenic element, or to (ii) the great phagocytic activity and increased development of the macrophage system, which is such a striking feature of the defence mechanism in malaria (Cannon and Taliaferro, 1931; Taliaferro, 1932; Sinton and Harbhagwan, 1935), or to (iii) both these factors. There is no doubt, however, that there is a very definite 'strain-specific' element in the tolerance in addition to this 'species-specific' one. The exact nature of the mechanism responsible for this protection is uncertain, but it is probably of the nature of an 'opsonin', acting in conjunction with the macrophages, or, perhaps, a 'strain-specific' sensitisation of the cells of the reticulo-endothelial system. The experimental malaria of monkeys is eminently suitable for a

study of this problem, the results of which may possibly suggest a method for artificial immunisation against the disease.

Some of our unpublished works show that, to produce any effective tolerance in monkeys, the defensive mechanism of the host must be stimulated by a considerable amount of parasitic substance, and that, possibly also, a time factor is an important element. It was found that infections, which were cut short and permanently cured by vigorous treatment started on the first day that parasites were detected, caused the development of no apparent tolerance to homologous superinfection performed about a month later.

This tolerance lasts as long as the infection persists in most instances, and has been called 'premuniton', or 'concomitant' tolerance. It appears to depend on the continued stimulation of the defensive mechanism of the host by the products of the parasitic infection (i.e., a vaccination). After the infection has been eradicated, the protection may persist for some time as a 'residual' tolerance, but may tend to disappear gradually. The duration of this 'residual' tolerance requires much further study, for it has a very important bearing upon the epidemiology and treatment of the disease in man. Evidence suggests that it may last longer after infection with some strains and species of *Plasmodium* than after others, and that multiple superinfections, both homologous and heterologous, tend to increase both its degree and duration.

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EXPERIMENTAL APPLICATION OF PARIS GREEN FROM AIRCRAFT.

BY

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[21st December, 1936.]

At the commencement of the anti-malaria campaign, which was carried out in the Delhi area during 1936, an offer was received from the authorities of the Aeronautical Training Centre of India Ltd. to co-operate in an attempt to control mosquito breeding by the application of paris green from aircraft.

There is an extensive area along the course of the Jumna river, known as the Bela, which is flooded annually when the river rises as the combined result of the monsoon rains and the melting of the Himalayan snows. It was thought probable that the only means of controlling mosquito breeding in this area would be by spraying larvicides from aircraft, and the offer was therefore accepted.

During the period in which the apparatus was being constructed and preliminary experiments carried out, it became obvious that the portion of the Bela which it was desired to control could be dealt with more satisfactorily and at less expense by other methods, and therefore aircraft dusting was never actually employed as part of the anti-malaria campaign. It is thought, however, that the following account of the preliminary experiments may be of interest to other workers.

The object of the experiments was to test the efficacy of the apparatus and the suitability of the diluent, which consisted of powdered soapstone obtained from the Sial Soapstone Mining Company, Barwara, Jubbulpore, C. P.

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The paris green used was supplied by Imperial Chemical Industries (India), Ltd., Calcutta.

The experimental flights were made over what is known as the 'brickfields area', situated immediately to the south of New Delhi. This consists almost entirely of excavations which are filled with water during the rainy season, and there is very little vegetation of any sort.

The aeroplane used was a DH 83 Fox Moth biplane, the property of the Himalaya Air Transport and Survey Co., Ltd. The dust was released from a hopper installed in the passenger cabin into a venturi tube mounted beneath the fuselage by means of a sliding valve controlled by wires from the pilot's cockpit. Fuller details regarding the aeroplane and the construction and working of the hopper and venturi tube are given in the Appendix.

After the first experiment the pilot experienced unpleasant after effects as the result of inhaling paris green dust. During the second experiment he wore an army anti-gas mask, and no ill effects resulted.

EXPERIMENT I.

Owing to an error in calculation, paris green was applied in this experiment at the rate of 2 lb. per acre, instead of 1 lb. per acre, as had been intended.

The area sprayed was roughly quadrilateral in shape and 35 acres in extent, and this was treated with 200 lb. of paris green mixture. The diluent was soapstone powder, and the proportion of paris green in this was 33 per cent. The wind was 10 to 15 miles per hour, its direction being from east to west.

The aeroplane was flown at a height of 50 to 100 feet, at an average speed of 70 miles per hour. The total flying period was 42 minutes, and the pilot flew from north to south, covering the area in four longitudinal strips, each of which was sprayed three times.

Twenty-four dishes, each containing 25 to 50 third or fourth stage anopheline larvæ, had been distributed over the area as shown in Diagram I. A glass microscopic slide, on which an area one inch square had been marked with a diamond, was fixed by means of plasticine across the corner of each dish.

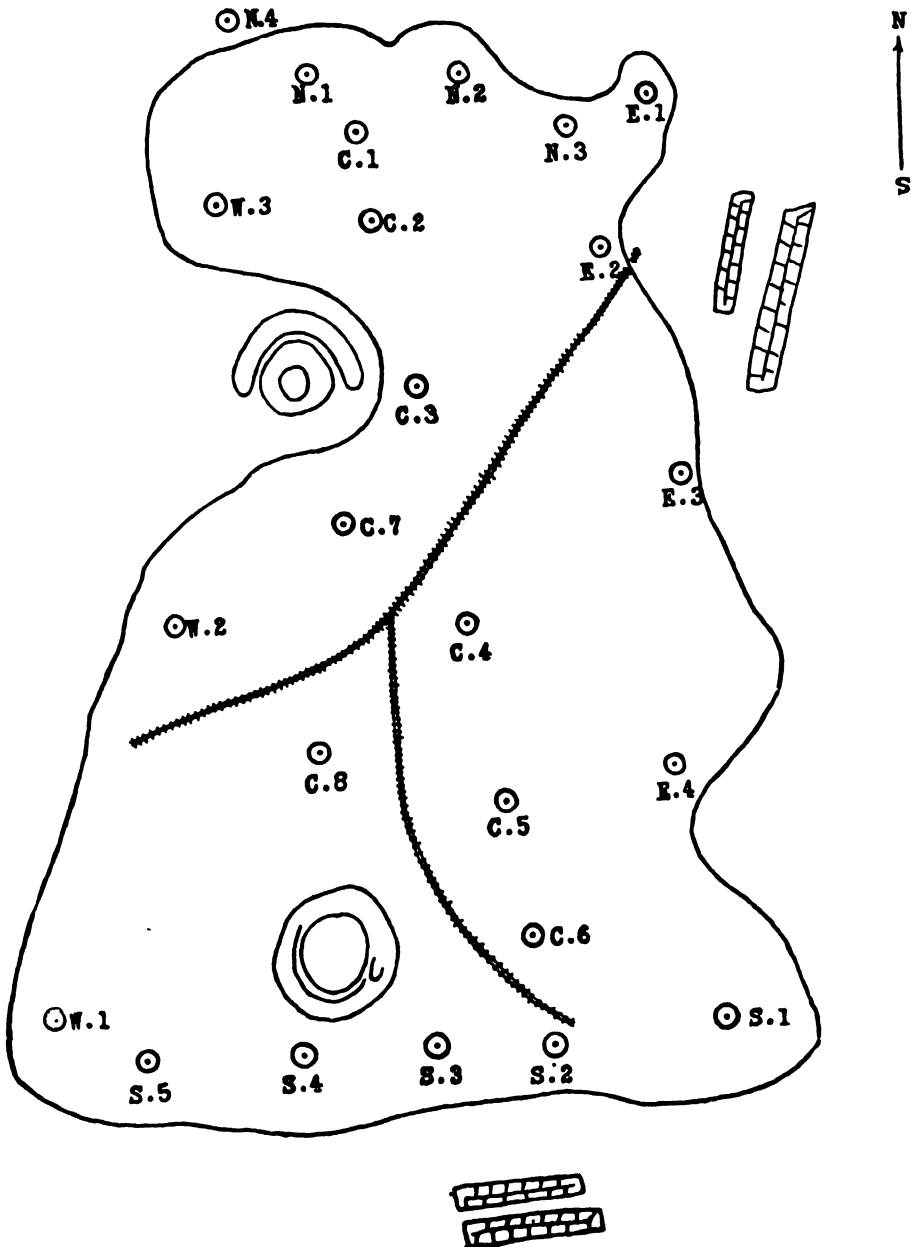
The percentage of larvæ killed in each dish, and the number of paris green particles per square inch counted on each slide, are given in Table I.

From a study of Diagram I and Table I, it is obvious that the extreme northern part of the area was almost completely missed, owing to the fact that the pilot failed to release the dust in time.

EXPERIMENT II.

In this experiment, the same area was again sprayed with a 33 per cent dilution of paris green in soapstone powder, but this time only 105 lb. of the mixture was used, so that the paris green itself was applied at the rate of approximately 1 lb. per acre. L-shaped strips of white muslin were placed at the four corners of the area, in order to give a better guide to the pilot.

DIAGRAM I



The wind velocity was 2 to 5 miles per hour, the direction being from west to east. The aeroplane was flown at a height of about 75 feet, and was

kept at a more constant altitude than during the first experiment. The flying period was 30 minutes, and the area was sprayed from north to south in two strips, the western receiving two dustings and the eastern three.

Three sets of observations were carried out during this experiment :—

(1) Twenty dishes, each containing 25 anopheline larvæ, were distributed over the area, each with a marked glass slide placed across one corner, as in

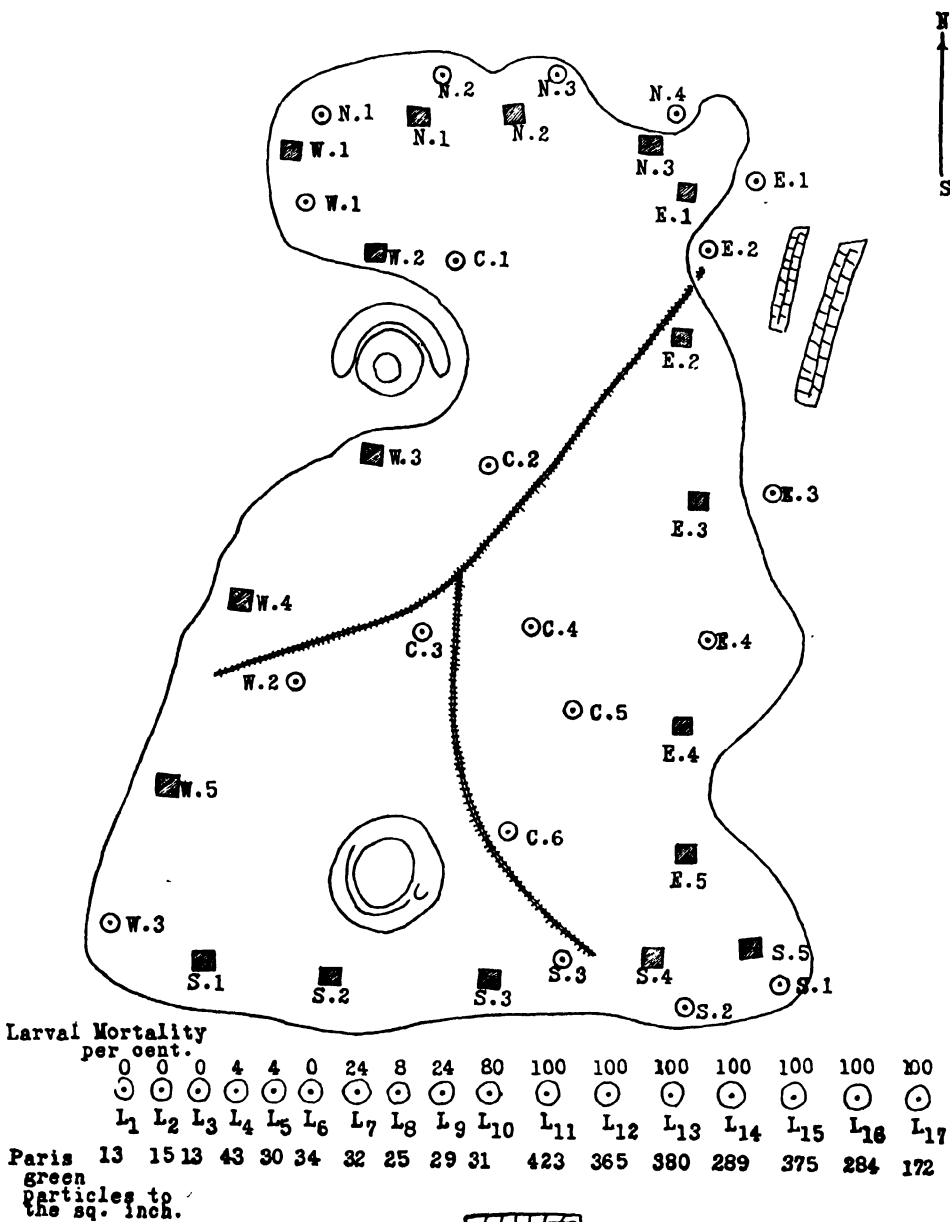
TABLE I.
(*Experiment I.*)

Number of dish.	Percentage mortality of larvæ.	Paris green particles per square inch.
NORTH BANK.		
N/1	0	10
N/2	0	9
N/3	0	2
N/4	0	2
EAST BANK.		
E/1	0	11
E/2	100	51
E/3	100	110
E/4	100	123
WEST BANK.		
W/1	100	29
W/2	100	..
W/3	100	37
SOUTH BANK.		
S/1	50	6
S/2	100	208
S/3	100	175
S/4	100	270
S/5	100	10
CENTRAL PORTION.		
C/1	100	150
C/2	100	85
C/3	100	71
C/4	100	102
C/5	100	52
C/6	100	39
C/7	0	12
C/8	100	103

Experiment I. The positions of these are shown in Diagram II, by circles marked N 1-4, W 1-3, S 1-3, E 1-4 and C 1-6. The results are shown in Table II.

(2) Seventeen dishes, each containing 25 larvæ, were placed at intervals of 20 yards along the southern bank, at right angles to the direction of flight of the aeroplane. These were intended to indicate the width of the lethal

DIAGRAM II.



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path, and each was covered by another large dish immediately the spraying over one strip was completed. The positions of the dishes are shown in Diagram II by circles marked L 1-17, and the percentage larval mortality in each dish and the number of particles of paris green per square inch are also shown.

(3) Four inspectors made a specific number of dips in five different situations along each of the four banks before spraying and again six hours after spraying, the average number of living larvæ per dip being recorded in this manner. The results are given in Table III, and the positions where dipping was carried out are indicated by shaded squares in Diagram II.

TABLE II.
(Experiment II.)

Number of dish.	Percentage mortality of larvæ.	Paris green particles per square inch.
NORTH BANK.		
N/1	40	21
N/2	100	42
N/3	100	27
N/4	100	281
EAST BANK.		
E/1	100	452
E/2	100	320
E/3	100	..
E/4	100	251
WEST BANK.		
W/1	4	12
W/2	0	11
W/3	0	10
SOUTH BANK.		
S/1	100	35
S/2	100	435
S/3	100	..
CENTRAL PORTION.		
C/1	100	180
C/2	100	125
C/3	100	325
C/4	100	125
C/5	100	248
C/6	84	19

A study of Diagram II and Tables II and III shows that the extreme western portion of the area was almost completely missed out, as was indeed obvious to observers on the ground who were watching the drift of the dust cloud. The results were entirely satisfactory over the remainder of the area.

As regards the lethal path, this could not be accurately determined, because the flights were made too far towards the east. Hence the eastern

limit of the lethal path could not be mapped out. All that can be said is that since the larval mortality was 100 per cent in seven consecutively placed dishes, the lethal path was at least 120 yards in width.

TABLE III.
(Experiment II.)

Place of dipping.	AVERAGE NUMBER OF LARVÆ PER DIP.	
	Before spraying.	After spraying.
NORTH BANK.		
N/1	6	0.0
N/2	19	0.6
N/3	10	0.4
Average	11	0.3
EAST BANK.		
E/1	8	0.0
E/2	44	1.0
E/3	45	2.2
E/4	7	0.2
E/5	37	0.2
Average	28	0.7
WEST BANK.		
W/1	1	0.0
W/2	2	1.0
W/3	50	52.4
W/4	25	23.4
W/5	6	0.2
Average	17	15.4
SOUTH BANK.		
S/1	40	5.2
S/2	28	2.6
S/3	28	2.2
S/4	20	0.0
S/5	50	0.8
Average	33	2.2

DISCUSSION.

Had it been decided that paris green dusting from aircraft was likely to prove of practical use in the anti-malaria campaign in the Delhi area, further experiments would have been carried out to determine which dilution of the larvicide would be most effective under varying conditions, and to give the pilot further experience in the technique of spraying. Our preliminary experiments showed that the soapstone powder employed was a very suitable diluent, and that the method resulted in the destruction of a high percentage of larvæ.

There are, however, certain disadvantages in this method of larval control, which became more and more obvious to us during the course of the experiments :—

(1) The method requires extreme skill and keenness on the part of the pilot, who indeed runs a very considerable risk, as he has to fly very low in order to get the best results.

(2) An extended series of experiments is necessary to determine the best technique for any given area. Although, when the technique is perfected a large extent of breeding area can be dealt with in a short time, yet it is not a method which can be applied in a fresh area at a moment's notice.

(3) There is always a risk that certain parts of the area may be insufficiently dusted on account of sudden changes in the direction and velocity of the wind.

(4) Unless an aeroplane and the services of a pilot can be obtained free of cost the method is more expensive than the application of larvicides by hand. The cost in any particular locality varies considerably, depending largely on the distance between the breeding ground and the aerodrome, to which the pilot must return at intervals to re-fill the hopper.

We are of opinion that the application of paris green from aircraft is only justified where the breeding area is very extensive, where it is essential to control such breeding regularly over an extended period, and where it is impossible to apply with success any other method of larval control.

In other countries the method has been used with success where there are extensive swamps near areas of naval or military importance in which dangerous malaria-carrying mosquitoes have been found breeding in large numbers; or in cases where dams have been thrown across valleys, forming reservoirs with hundreds of miles of shore-line, where important centres of population are affected, and where hand labour is very costly.

All things considered, we do not think that in the present circumstances it is a method which is likely to prove of practical value in India.

ACKNOWLEDGMENTS.

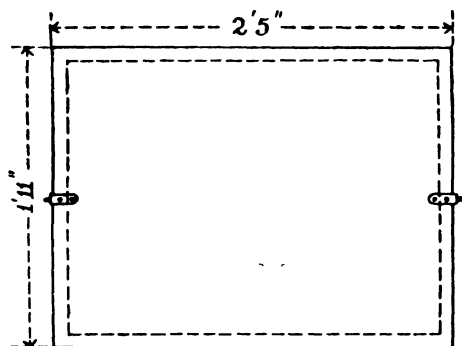
We wish to tender our thanks to Surgeon L. L. Williams Jr., United States Public Health Service, for kindly supplying photographs and descriptions of aircraft dusting equipment used in the U. S. A.; to Captain A. T. E. Eadon, F.R.A.E.S., M.I.A.C.E., Principal, Aeronautical Training Centre of India, for his co-operation in carrying out the experiments; to Major E. B. Brasier-Creagh, M.C., Chief Staff Officer, Aeronautical Training Centre of India, under whose direction the hopper and venturi tube were constructed and installed in the aeroplane; and to Cadet-Captain J. D. Mody, the pilot of the machine, who displayed the utmost keenness in making the experiments a success.

APPENDIX.

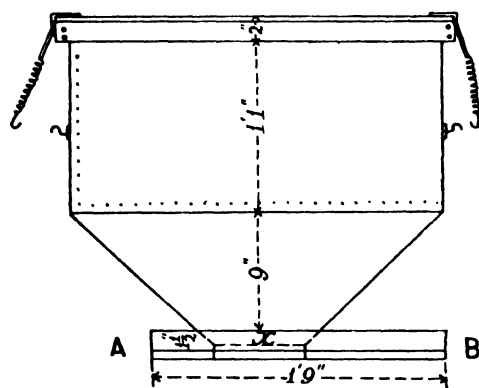
(Extracted from a note kindly supplied by Major E. B. Brasier-Creagh, M.C., of the Aeronautical Training Centre of India.)

The machine used was a DH 83 Fox Moth. Its payload, exclusive of petrol, oil and pilot, worked out to almost 510 lb.

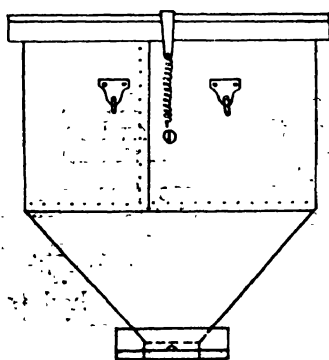
HOPPER.



PLAN.



FRONT ELEVATION.



SIDE ELEVATION.

Fig. 1.

In the two-door cabin of the Fox Moth, designed to hold three persons, there was a floor space of 36 by 17 inches extending across the centre of gravity. In front of this space was a transverse floor member about one inch square, and at the back projected forwards the cover of the aileron operating mechanism, occupying some 10 by 5 inches of ground area. The maximum height of this cover was less than 5 inches.

The hopper was constructed of 18 gauge galvanised iron of the dimensions shown in Fig. 1. It was provided with a cover of wood, the returns of which were chamfered off to correct any distortion that might occur in the top edge. The top was fixed by two spring clips. The nose of the hopper lay in a female bed lined with felt which was fixed to the floor of the aircraft, and the whole hopper was held in position by four guy wires running from it to the bottom side members of the fuselage.

Immediately below the floor was fixed a plain sliding valve with a total run of 8 inches, consisting of a 16-gauge iron plate sliding between two similar plates seated in wood. The opening was 8 by 4 inches, and lay transverse to the fore and aft axis of the aircraft.

Control of the valve was effected by flexible wires passing over pulleys attached near the base of the lower wings, and running therefrom directly through the floor and cabin back wall, one to each side of the pilot's cockpit. In the final design the opening wire ended as a rod with pistol butt grip running between fibre bearing surfaces, in order that the pilot might adjudge the various degrees of opening; the closing wire was simply fitted through a small length of aluminium piping and turned on itself so as to provide a rough hand grip. Below the valve a plain conduit led into the upper surface of the venturi tube (Fig. 2).

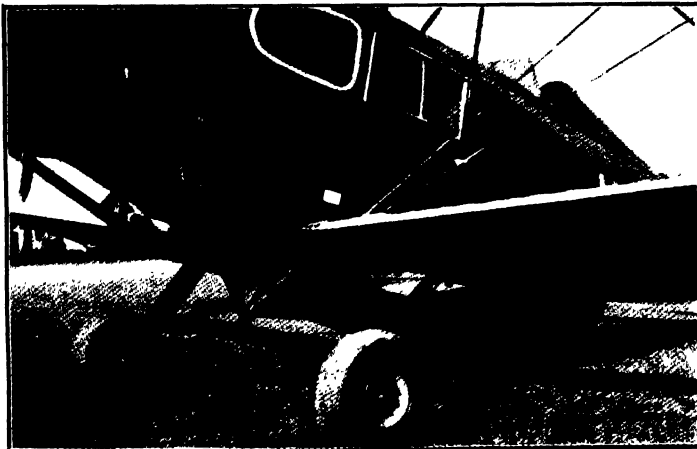
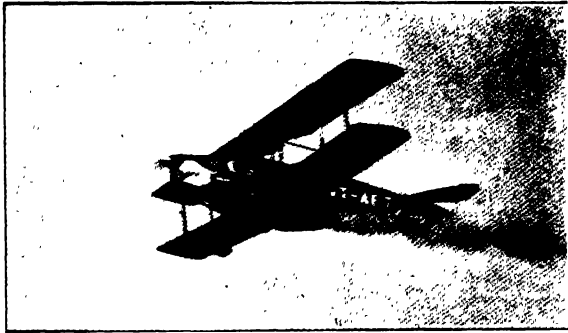
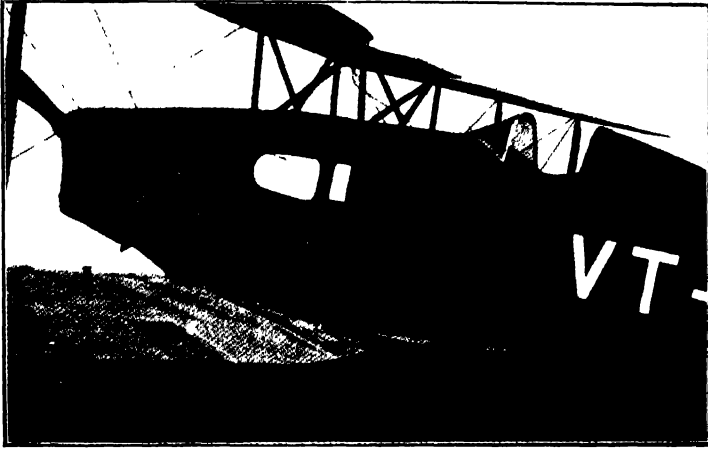
The construction of the venturi tube presented a great many problems, in the solution of which the designs available rendered little assistance.

A commencement was made at the triangular upper member of the under-carriage, and the tube was given a triangular form as far as its constriction, which coincided with the forward edge of the conduit. The triangle was inverted, with its flat surface upwards for the reception of the conduit. Behind the entry of the conduit this flat upper surface was expanded to the full width of the fuselage up to and slightly behind the pilot's cockpit. By the insertion of horizontal triangular pieces at the bottom angle of the triangle, the venturi tube was gradually altered in sectional shape from the original triangular form at the constriction to a rectangular form at its outlet end, and the walls were warped to join together the upper and lower members.

The interior angles of all surfaces were strengthened with continuous angleblocks and the whole interior was finally lined with fabric and doped. Being constructed with three-ply wood the venturi tube, when finally gripped at intervals along the outer angles by short strips of iron, became extremely firm, and had the additional merit of elasticity. It was found that the whole equipment could be detached from and attached to the machine in less than one hour, and in order to keep this timing as low as possible no attempt was made to fair off any of the exterior equipment.

It was not found necessary to use any form of agitator, and less than half a pound of dust remained in the hopper at the completion of a flight. On the ground, with full engine and the valves fully open, the dust was observed

PLATE I.



to enter the venturi tube in a very steady flow, but the cloud at the outlet appeared in waves, apparently caused by the eddy formed by the passage of the propeller arms past the venturi tube opening. The wavy form of the cloud maintained its appearance during flights as well. Although a small deflector was fitted to the upper side of the outlet, the cloud was not kept

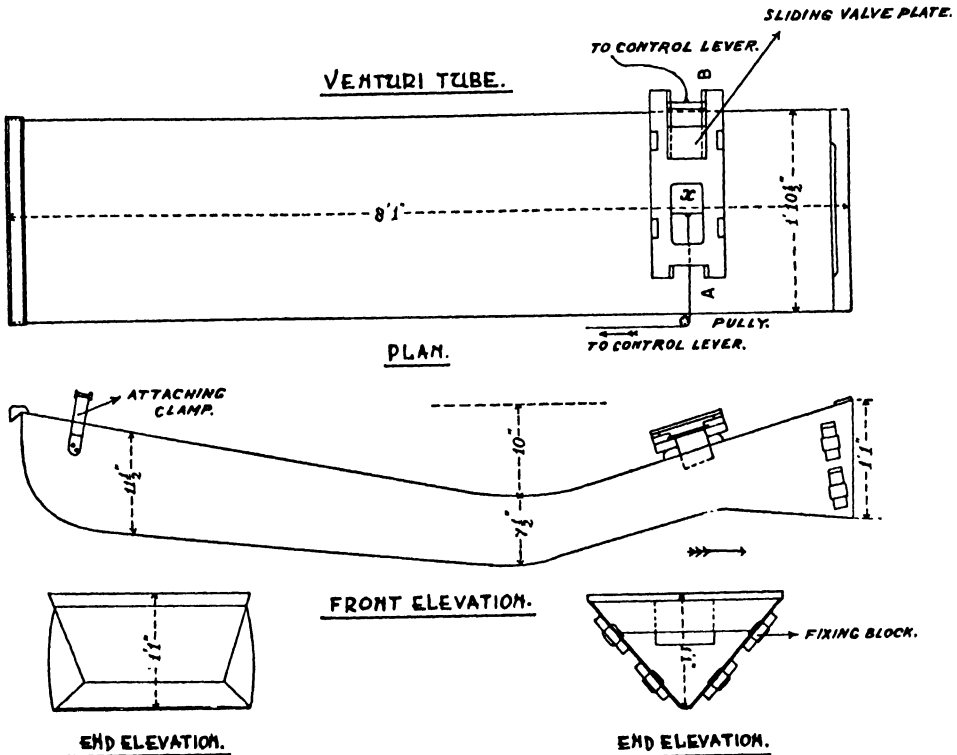


Fig. 2.

clear of the lower tail members of the aircraft, and the forward surfaces of all parts below the elevating plain collected considerable quantities of paris green. It is considered that the curved form of the venturi tube, necessary in order to keep it clear of the aileron controls, is the cause of this upward flow.

The aircraft, which originally had a stalling speed of about 60 miles an hour, stalled after the fitting of the equipment with full hopper at 65 miles per hour, and commenced to lose height at 70 miles per hour. The installation did not appear in any way to affect the other aerial properties of the aircraft.

AN IMPROVED TECHNIQUE FOR MARKING AND CATCHING MOSQUITOES.

BY

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(Indian Research Fund Association.)

[23rd December, 1936.]

IN order to determine the behaviour of mosquitoes under natural conditions, a large number of workers have utilised various methods of staining and marking these insects. These are so well known that their description is unnecessary. In the course of an experiment now in progress at the Ross Field Experimental Station for Malaria at Karnal, a technique was evolved for marking mosquitoes which offers considerable improvement over those previously used.

The procedure is to stun a batch of, say, 20 mosquitoes in a test tube by means of a short exposure of not more than 3 seconds to the effects of a drop of chloroform or æther, placed on the cotton-wool plug. The mosquitoes are then identified and counted and transferred to a glass chimney through the central hole in a cardboard cover placed over one end (*vide* Fig. 1), while its other end is previously secured with a piece of netting. When the mosquitoes begin to display signs of regained activity, which normally takes about 20 minutes, the cardboard cover is also replaced by netting. The chimney prepared in this manner has an average capacity of 200 mosquitoes, which should not be exceeded.

The actual marking is carried out by means of the dusting pump illustrated in Fig. 1 (c). The pump is of simple construction and can be readily prepared in a laboratory. It consists of an entomological specimen tube 3 inches \times 1 inch provided with a well fitting rubber stopper, bearing two short curved pieces of glass tubing of small bore as shown in Fig. 1. One of these tubings is connected with a rubber pump of the Higginson's enema syringe variety while the other acts as the outlet tube for delivering the dust.

The specimen tube is filled with the marking powder known as 'Gold' powder commonly used in press works for decorative writing. This powder is light and inert and does not harm the mosquito. Its greatest advantage is that it does not stick to the wings of the mosquito and thus presumably does not interfere with its flight. It is cheap and is available in a variety of other colours such as silver, green, red, etc., each of which can be employed for differentiating a particular batch. It is unnecessary to use too much of the powder; enough to cover the thorax and abdomen has been found satisfactory. Immediately after dusting the mosquito will be observed to manipulate its legs and wings in an endeavour to remove the dust but sufficient always remains for effectual identification later.

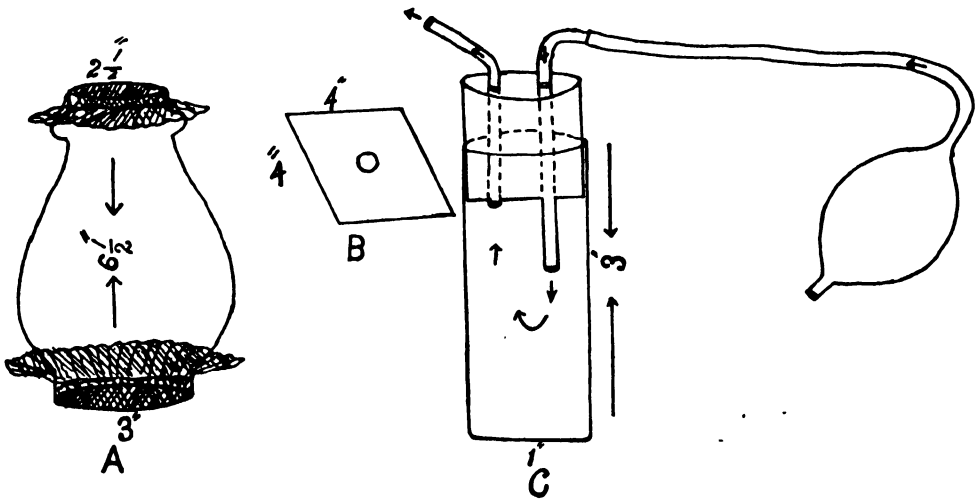
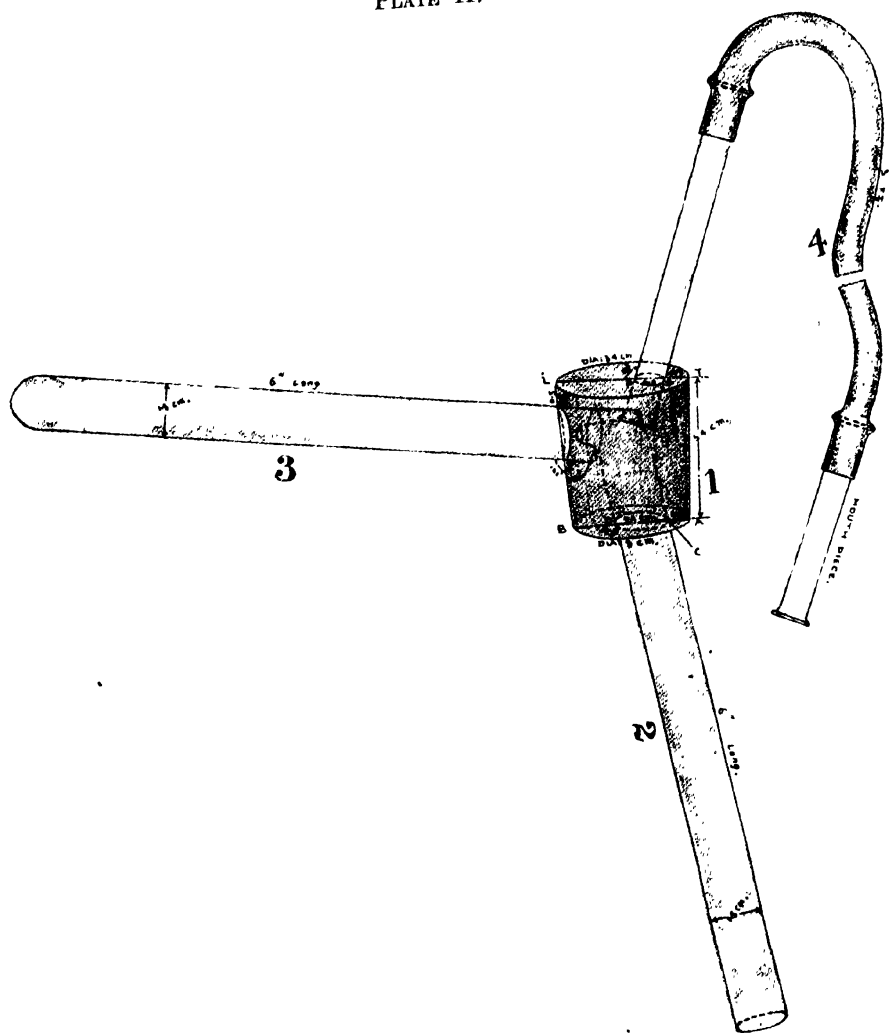


Fig. 1.

The examination of the recaptured mosquitoes for evidence of dust can be very rapidly carried out. The mosquitoes are lightly chloroformed in batches and transferred to a watch glass for examination under a binocular dissecting microscope. With the help of a needle the mosquitoes are gently stirred and exposed to view when the dusted ones can be readily identified due to the conspicuous metallic lustre of the powder particles. A dusted mosquito bears a large number of particles of powder which form distinct bands in the region of the sternites, the neck and the coxæ. They should not be confused with some specimens that may be covered with occasional particles acquired during collection through contact with the dusted ones.

After the examination, the mosquitoes are transferred to the glass chimney where they soon recover and can be subjected to further experimentation. The mosquitoes thus need not be killed for purposes of identification and herein lies the chief advantage of this method.

PLATE II.



Mosquito catching apparatus.

MOSQUITO CATCHING APPARATUS.

This consists of three glass tubes fitted into a rubber stopper. The specifications of the component parts are noted in Plate II and their details are as follows:—

1. *The rubber stopper* (Plate II, fig. 1).—This has the following dimensions:—
Height 3·4 cm.; diameter of the top flat surface 3·4 cm., of the lower flat surface 3·0 cm.
2. *The catching tube* (Plate II, fig. 2).—This is an ordinary test tube about 1·4 cm. in diameter. The lateral surface of its blind end is drawn out sideways into a projection which is perforated with an opening large enough to afford easy passage to a mosquito. This end is inserted into the lower flat surface of the stopper at an angle of 100°.
3. *The receiving tube* (Plate II, fig. 3).—This is also a standard-sized test tube 1·4 cm. in diameter, the open end of which is fitted into the curved lateral surface of the stopper at an angle of 100°. Into this tube is directed the perforation of the catching tube described above.
4. *The suction tube* (Plate II, fig. 4).—This is a piece of glass tubing 0·8 cm. in diameter inserted into the top flat surface of the stopper at an angle of 110°. Its inner end rests above and close to the catching tube while its outer end is connected with a rubber tube bearing a glass mouth piece.

The catching tube and the suction pipe remain permanently fixed into the stopper, and the receiving tube is fitted only at the time of catching. Slight suction is applied every time the catching tube is brought near a mosquito, when the latter is drawn into the receiving tube where it is trapped. The receiving tube is changed after about 20 mosquitoes have been collected.

The chief advantage of this apparatus lies in the rapidity with which resting as well as flying mosquitoes can be collected, and even those hiding inside the crevices are not outside its catching range. Another feature of the apparatus is that it is almost mechanical in its action and its use therefore would greatly eliminate the human element from the results of the catching station. Finally the number of test tubes required for a collection is reduced, as the receiving tube need only be changed after catching 20 or more mosquitoes.

I wish to express my gratitude to Lieut.-Colonel G. Covell, I.M.S., Director, and Major M. K. Afridi, I.M.S., Assistant Director, Malaria Survey of India, and Dr. E. P. Hicks, M.D., for affording me the necessary facilities and encouragement.

I am also thankful to laboratory assistant A. David and to the other laboratory staff for their whole-hearted co-operation.

AN INVESTIGATION INTO THE MOSQUITOCIDAL VALUE OF INDIGENOUS DERRIS AND OTHER DRUGS.

BY

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[2nd January, 1937.]

IN the case of anopheline mosquitoes which usually pass a considerable portion of their lives in houses, the use of an effective insecticide may be of considerable value as an anti-malarial measure, apart from any considerations of the comfort of the inhabitants. The extent to which the infected mosquitoes remain indoors may vary considerably with the species, but there is a strong possibility that a number of potentially infective insects may linger in dwellings for at least some hours after obtaining a blood meal. In this connection a quotation from the work carried out in the Union of South Africa and reported by Thornton (1935) is of great interest. He says :

‘We have been driven to employ hut spraying during the malaria season as our main weapon over extensive sections of the country, and after a two-years’ trial on the large scale are satisfied that the disease can be controlled by it in Natal and Zululand. It is essential, however, that (a) hut spraying should commence as soon as adult *gambia* or *funestus* begin to enter dwellings; (b) it be applied to every habitation over the area to be dealt with, and that such area be as large as possible; (c) every dwelling be thoroughly sprayed at least once weekly, but preferably bi-weekly.....our method can only be successful when the vectors are in the habit of frequenting houses. Reduction in the infectivity rate may be very marked’.

Where breeding is very restricted, or cost is a minor consideration, anti-larval measures have proved very effective. In Natal and Zululand there are tracts inhabited by a scattered population where anti-larval measures are very

difficult and the cost of their thorough application is prohibitive. In parallel experiments carried out in two areas, adopting mosquito spraying in the one and anti-larval measures in the other, it was found that anti-adult work cost only about a third of the anti-larval work and was more effective (*vide* Annual Report of the South African Institute for Medical Research, December 1935, page 30).

Similar conditions are obtainable in several parts of India, where methods for larval destruction are not applicable and the spraying of huts may play an important part amongst the anti-malarial measures. This is not a plea for the exclusion of anti-larval measures, but a justification for hut spraying where the domestic habits of the anopheline mosquitoes demand it or where finances do not permit the adoption of anti-larval measures.

Although a fair amount of work has been carried out on the mosquitocidal value of various chemicals and drugs, the fact remains that so far with the exception of pyrethrum flowers, no other source fulfils the conditions expected from an ideal insecticide. It was hoped that Derris root extracts would replace, at least partly, pyrethrum solutions for killing flies, mosquitoes, etc., but this optimism was rather premature, as lately experimental work has shown Derris root to be a contact and stomach poison requiring direct spraying on the insects. The primary object of this investigation was to explore the possibility of evolving some efficient formulæ which an Indian villager may prepare in his own house from ingredients easily accessible and within the reach of his pocket.

The desiderata of an ideal mosquitocidal spray have been discussed by Sinton and Wats (1935), who have also laid down a standard biological method for assaying the value of insecticidal spraying solutions. Keeping these requirements in mind and using the same technique, we have explored various sources to find an efficient solution.

METHODS OF EXTRACTION.

Different methods were employed for the isolation of the active principles of the drugs under experiment, viz., alcoholic, ethereal, chloroform and kerosene oil extraction.

(1) ALCOHOLIC EXTRACTION.

Alcoholic extraction has been based on the lines of the British Pharmacopœia (1932) and, where available, the B. P. tinctures themselves were used. These extracts were used as such for testing because a blank experiment with alcohol alone proved to be non-lethal to mosquitoes.

(2) ETHEREAL AND CHLOROFORM EXTRACTS.

The appropriate quantity of the drug was extracted in a Soxhlet apparatus from 4 to 8 hours. The extract was filtered, when necessary, and allowed to evaporate at a low temperature. The residue was dissolved in kerosene oil to a suitable strength for testing. In some cases where kerosene oil solutions were not clear the residue was first dissolved in a few c.c. of chloroform or acetone and kerosene oil added. In these latter cases, a blank experiment was performed to demonstrate the non-toxic properties of chloroform or acetone-oil mixture.

(3) KEROSENE OIL EXTRACTS.

Some drugs were extracted in kerosene oil by leaving the powder or paste in contact with the oil for seven days, with daily shaking of the receptacles. In some cases the chemicals were directly incorporated in the kerosene oil and used for testing.

TESTING THE TOXICITY OF EXTRACTS OR MIXTURES.

For this purpose, the method of Sinton and Wats (1935) was employed. One c.c. of the undiluted test solution was sprayed with a No. 15 De Vilbiss sprayer into the test chamber. Twenty-five anopheline mosquitoes (*A. annularis* or *A. subpictus*) were introduced into the chamber and collected after 30 minutes' exposure. These insects were then put in a mosquito hotel (Sinton, 1934) and placed in a dark room for 24 hours under same conditions as a number of control insects. The results were read immediately after collection to note the number of insects flying, and after 24 hours to record the numbers dead and moribund.

RESULTS.

The results in all cases have been much inferior to those recorded with the standard mosquitocide (1 c.c. of 1 per cent solution of pyroicide 20 in kerosene oil). Owing to the low lethal powers of the solutions tested, too many details have not been given.

The list of drugs and other chemicals together with the modes of extraction and concentration used are shown in the table at the end of the paper.

DISCUSSION.

Plants which are toxic to fish have been tried as insecticides and found to give hopeful results in agricultural and some horticultural work. Derris can be taken as an example. The root has been used for a long time by the inhabitants of Malaya, India and the Philippine Islands as a fish poison. The root is an imported article of trade for agricultural and husbandry purposes, being a contact and stomach poison for several species of insect pests. *Derris elliptica* and *Derris malaccensis* are the chief sources, being obtained chiefly from Malaya, Sarawak, British North Borneo and the Dutch East Indies. Several species are known to occur in India, of which *Derris robusta* and *Derris scandens* were examined by McIndoo, Sievers and Abbott in 1919 and found to be devoid of insecticidal properties. *Derris uliginosa*, which occurs in Bengal and Assam, was examined by Tattersfield in 1926-27 and was found to possess meagre insecticidal properties. Uichanco (1933) has written a monograph on the three species of Philippine Derris and gives a valuable summary regarding the insecticidal value of the drug. Krishna and Ghose (1936) have investigated the rotenone and allied insecticidal principles of the Indian Derris roots. *D. elliptica*, which was not known to occur in India, has been found growing in Assam and examined along with others for its chemical constituents. The total ethereal contents of the Indian species are very low; hence a sample even though containing 2.5 per cent of rotenone has to be classified as poor from the commercial point of view.

Our investigation has been limited to the investigation of mosquitocidal values only and we are rather disappointed. This was anticipated to some extent, as Derris is a contact poison, but even with a 40 per cent solution of

an ethereal extract, when sprayed into the test chamber before and after introduction of the mosquitoes, the mortality was only about 60 per cent. To control this experiment we have similarly used the ether soluble parts of Derris (containing 5 per cent rotenone), obtained from a reliable American firm, but found it to be no better.

There are other fish poisons in the table which have similarly given poor results. One per cent emulsion of croton seeds used for fishing in South India and walnut shell (green) used in the Simla Hills was very effective in the laboratory against *Gambusia* fish, but proved to be useless for killing adult mosquitoes. We infer from our experiments that insecticidal and piscicidal values do not go hand in hand. It may be more reasonable to suppose that the fish poisons may serve as larvicides. Croton oil emulsions are being investigated by us for the purpose and it is hoped to publish the results in the near future.

The extracts of Indian chrysanthemum flowers were found to be of some value, but to get a 100 per cent kill very strong concentrations would be required and would not prove cheap enough for the purpose. Amongst the group of miscellaneous chemicals some seemed to be effective for stunning the insects, but were too pungent to be of any practical value.

CONCLUSIONS.

(1) Sprays prepared from 65 different sources have been tested and found to be poor for killing adult mosquitoes.

(2) Several of these drugs were well-known fish poisons, hence it follows that mosquitocidal and piscicidal values may not go hand in hand.

ACKNOWLEDGMENTS.

We desire to record our thanks to the Directors of the Malaria Survey of India, Kasauli, and Haffkine Institute, Bombay, for the facilities afforded to carry out the inquiry. We are grateful to Dr. S. Krishna and Mr. T. P. Ghose of the Chemical Branch, Forest Research Institute, Dehra Dun, for supplying the ethereal extracts of the various species of Indian Derris and some other drugs reputed to have insecticidal values. We are indebted to Father J. F. Caius, St. Xavier's College, Bombay, for having corrected the botanical names, and to Mr. A. S. Narasimham, Assistant Technician, Haffkine Institute, Bombay, for his valuable assistance.

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TABLE.

Name of the drug.	Common Hindi name.	Part used.	Concentration used (per cent).
ALCOHOLIC EXTRACTS.			
<i>Aconitum napellus</i> Linn.	Bachnag	Root	10
<i>Adhatoda vasica</i> Nees.	Arusha	Leaves	10
<i>Atropa belladonna</i> Linn.	Sag-angur	"	10
<i>Cannabis sativa</i> Linn.	Ganja	Flower tops	10
<i>Cantharis</i>	"	B. P. tincture	1 in 10,000
<i>Cassia angustifolia</i> Vahl.	Senna	Leaves	20
<i>Psychotria ipecacuanha</i> Stokes	"	Roots	10
<i>Colchicum autumnale</i> Linn.	Surinjan	Seeds	10
<i>Datura stramonium</i> Linn.	Dhatoora	Leaves	20
<i>Eucalyptus globulus</i> Labill.	Karpura-maram	"	5
<i>Ferula foetida</i> Regel.	Hing	Oleo-gum resins	20
<i>Hyoscyamus niger</i> Linn.	Khorasaniajowain	Leaves	10
<i>Podaphyllum emodi</i> Wall.	Papra	Resin	3.65
<i>Rhus toxicodendron</i> Linn.	Kakarsingi	Leaves	50
<i>Strychnos nux-vomica</i> Linn.	Kuchla	Seeds	10
ETHEREAL EXTRACTS.			
<i>Anona squamosa</i> Linn.	Sitaphal (Shari-fah).	Leaves	50
<i>Calotropis gigantea</i> Ait.	Madar (Akra)	Root	10
<i>Chrysanthemum indicum</i> Linn.	"	Yellow flowers	5
		Mauve flowers	5
<i>Deris elliptica</i> Benth.	Kajarwel	Root	40 †
" <i>scandens</i> Benth.	"	"	10 †
" <i>uliginosa</i> Benth.	Kajarwel	* Crys. subst.	5 †
<i>Euphorbia nerifolia</i> Linn.	Thohar	Root	10 †
<i>Garcinia morella</i> Desr.	Tamal	Leaves and stalk	10
<i>Herpestis monniera</i> H. B. and K.	Brahmi	Resin	5 †
<i>Millettia pachycarpa</i>	"	Leaves and stalk	10
<i>Mullelea suberosa</i> Benth.	"	Root	10
<i>Pterospermum acerifolium</i> Willd.	Kamar	"	5 †
		Leaves	10
		Flowers	10
<i>Suertia "chirata</i> Buch-Ham.	Charayatah	Leaves and stalk	10
<i>Tephrosia hamiltonii</i> J. R. Drumm.	Sarphunkha	Root	5 †
" <i>purpurea</i> Pers.	"	"	5 †
" <i>villosa</i> Pers.	"	"	5 †
<i>Vitis "setosa</i> Wall.	Harmal	"	10
CHLOROFORM EXTRACTS.			
<i>Adhatoda vasica</i> Nees.	Arusha	Leaves	50
<i>Anona squamosa</i> Linn.	Sitaphal	Seeds	50
<i>Datura fastuosa</i> Linn.	Dhatoora	Capsules	50
	"	Leaves	50
<i>Girardinia "palmata</i> " Gaudich	"	"	30
<i>Juglans regia</i> Linn.	Walnut (Akhrot)	Green shells	20
		Bark	5
<i>Lantana camara</i> " Linn.	Ghaneri	Leaves	50
" " "	"	Roots	50
" " "	"	Flowers	50

* M. P. of the crystalline substance 274°C. to 275°C.

† As the mosquitoes were found active and alive after the usual 30 minutes' period, 1 c.c. more of the drug was sprayed to test the efficacy of the spray as a contact poison, but this did not improve the results to any extent.

114 Mosquitocidal Value of Indigenous Derris and Other Drugs.

TABLE—concl'd.

Name of the drug.	Common Hindi name.	Part used.	Concentration used (per cent).
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CHLOROFORM EXTRACTS—concl'd.

<i>Thevetia nereifolia</i> Juss.	.. Pila-kaner	Flowers	10
" " " "	.. " "	Leaves	10
<i>Uraria picta</i> Desv.	.. Dabra	" "	50
" " " "	.. " "	Stem	50

KEROSENE OIL EXTRACTS AND MIXTURES.

<i>Boenninghausenia albiflora</i> Reichb.	Pismari	Plant	50
<i>Cinnamomum camphora</i> T. Nees. and Eberm.	Kakronda	Camphor	1
<i>Nicotiana tabacum</i> Linn.	Tambaku	Leaves	55
<i>Semecarpus anacardium</i> Linn. f.	Bhela (Bibba)	Nut (powdered)	2

OILS, ETC.

<i>Brassica juncea</i> Coss. (seeds)	Rai	..	50
<i>Eugenia caryophyllata</i> Thunb.	Laung	..	10
<i>Chenopodium ambrosioides</i> Linn.	Bather sag	..	10
<i>Croton tiglium</i> Linn.	Jamalgota	..	1
<i>Cinnamomum zeylanicum</i> Nees.	Dalchini	..	10
<i>Gaultheria fragrantissima</i> Wall.	2
<i>Pinus longifolia</i> Roxb.	Saral, Chir	..	25

MISCELLANEOUS CHEMICALS (kerosene oil solutions).

Acid salicylic	1
Benzol trichloride	5
Bromine	Saturated
Carbon tetrachloride	2
Chlorine	Saturated by bubbling.
Iodine	1
Naphthaline	10
Quinine sulphate	1
Thymol	10

THE USE OF *GAMBUSIA AFFINIS* IN INDIA.

IN an article by Prashad and Hora*, which appeared recently in this journal, it was stated that *Gambusia affinis* were introduced from Siam about five years ago, and that they are available from the experimental station of the Malaria Survey of India at Karnal.

As a matter of historical interest, it should be stated that a consignment of *Gambusia* was brought to Bangalore from Italy in the autumn of 1928 by Dr. B. A. Rao of the Mysore State Department of Health, through the courtesy of Dr. L. W. Hackett of the Rockefeller Foundation. They have been used extensively in the State, and specimens have been supplied to many localities in India. It is obviously more convenient for workers in South India to obtain specimens from Mysore than from Karnal.

The *Gambusia* which formed the nucleus of the Karnal stock were received from Siam in 1929, to which country we believe they had also been introduced by Rockefeller Foundation workers.

Editor.

* PRASHAD, B., and HORA, S. L. (1936). A general review of the probable larvivorous fishes of India. *Rec. Mal. Surv. Ind.*, 3, 4, pp. 631-648.

CHOLESTEROL AND LECITHIN IN MALARIA.

BY

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[25th November, 1936]

ALTHOUGH it is recognised that the metabolism of cholesterol and lecithin is still imperfectly understood, the behaviour of these substances in the blood has been investigated in many pathological conditions as well as in normal subjects. It seemed reasonable to expect that the estimation of the cholesterol and lecithin content of the blood in malaria might disclose some interesting deviations from normal, since considerable changes in the blood cholesterol and lecithin have been shown to occur in such conditions as acidosis, jaundice, and protein shock, conditions with which the malarial paroxysm is believed to be closely related.

A search of the literature has revealed that the behaviour of the blood cholesterol and lecithin in malaria has received relatively little attention. A series of observations was, therefore, undertaken to determine the amount of cholesterol present in the peripheral blood at different stages of both human* and monkey malarial infections. Observations on lecithin were made in monkey malaria only.

METHODS OF RESEARCH.

The experiments to be described were designed to ascertain the cholesterol content of the blood before, during, and after the malarial paroxysm, and also after the completion of specific treatment, in as many cases as possible†.

Unfortunately, in human malaria it was not possible to make a continuous series of observations, in most of the cases, at all stages of the disease. In many instances, when estimations were made during the afebrile period, no

* Observations on human malaria were completed in 1931.

† In the majority of instances blood was taken for cholesterol estimations from the same patients, and at the same times, as for the estimations of blood sugar. The results of the latter observations have already been set forth by Sinton and Kehar (1931).

pyrexia occurred before it was necessary to institute specific treatment, while in other cases the patients were seen for the first time during the febrile stages, or even after the pyrexia had subsided.

In the case of monkeys, the cholesterol estimations were made during the normal, incubation, and patent infection periods till death supervened or recovery was complete.

The technique for the estimation of cholesterol in the blood described by Leiboff (1924) was followed, except that a metal ring was used to hold the circular piece of the extraction thimble in place, thereby facilitating complete extraction.

RESULTS OF THE INVESTIGATION.

A. OBSERVATIONS ON HUMAN MALARIA.

A series of cholesterol estimations was made on apparently healthy individuals living under the same conditions as the malaria patients examined. Thus in 48 British soldiers in Kasauli the average cholesterol level was 155.3 mg. per 100 c.c. plasma (maximum 180 : minimum 121)*. Among 45 healthy Indian prisoners in the Central Jail, Lahore, the average figure was 150.06 mg. per 100 c.c. plasma (maximum 192 : minimum 128).

The more complete observations on malaria cases were carried out at the Malaria Treatment Centre, Kasauli, among British soldiers suffering from chronic infections with *P. vivax* or *P. falciparum*. At the time of the first cholesterol estimations these patients had had no relapse for some time, nor had they received any specific treatment for malaria for a considerable period, usually several weeks.

The cholesterol estimations were carried out as follows :—

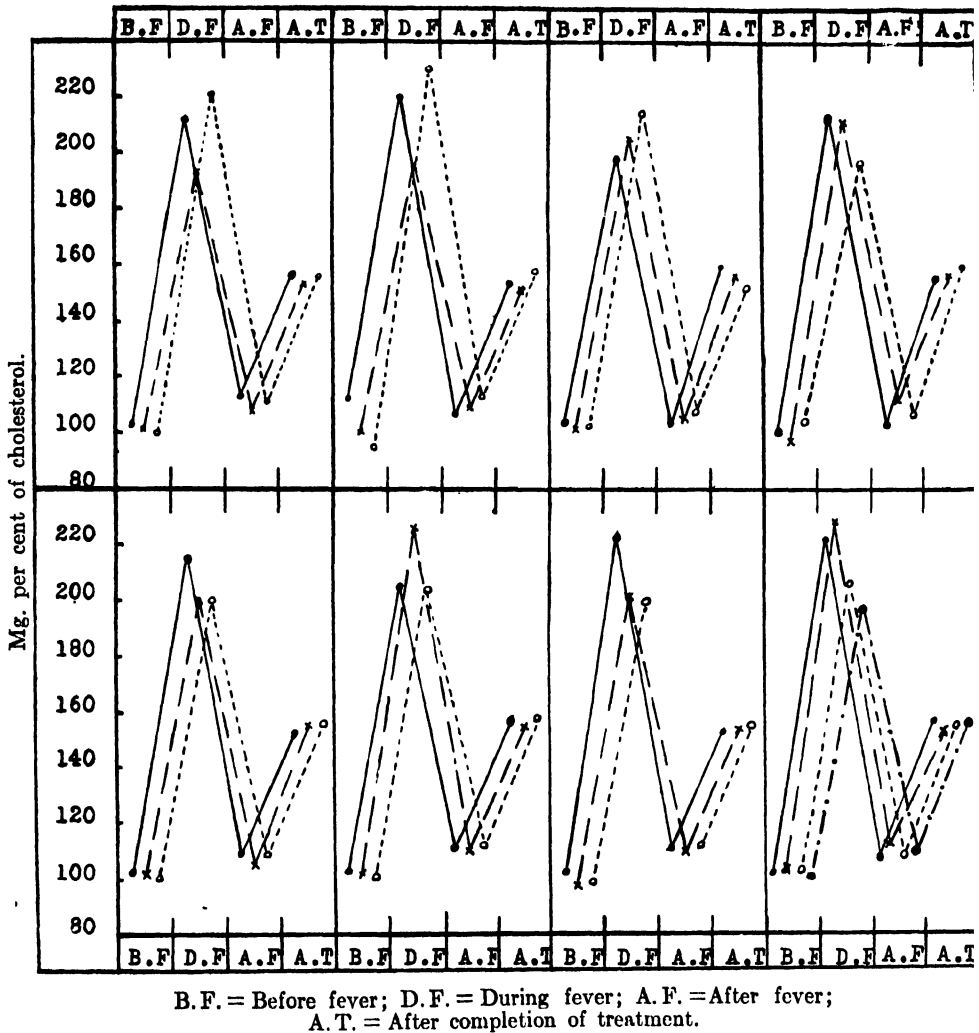
- (1) At the time of the appearance of parasites in the peripheral blood.
- (2) During the pyrexial period.
- (3) In the afebrile period immediately following pyrexia.
- (4) On the completion of specific treatment (usually a course of combined quinine and plasmoquine treatment lasting three weeks in benign tertian, and one week in malignant tertian malaria).

In 25 patients suffering from infections with *P. vivax* it was found possible to estimate the blood cholesterol in all the four stages mentioned above. In 14 of these cases the original estimations were made within about five hours of the onset of fever, and in the remainder within about 24 hours. The post-pyrexial observations were made within about 12 to 18 hours after the temperature had reached normal.

The results of these estimations have been shown graphically in Text-figure 1. The average figure for blood cholesterol in the pre-pyrexial stages was 100.68 mg. per 100 c.c. plasma (maximum 104 : minimum 95); in the febrile stage the average figure was 210.03 (maximum 235 : minimum 197); in the post-pyrexial stages 108.04 (maximum 113 : minimum 105); and after the completion of treatment 155.5 (maximum 160 : minimum 102).

* All cholesterol estimations were made in duplicate, and the figures represent the observed value in milligrams per 100 c.c. plasma.

TEXT-FIGURE 1.

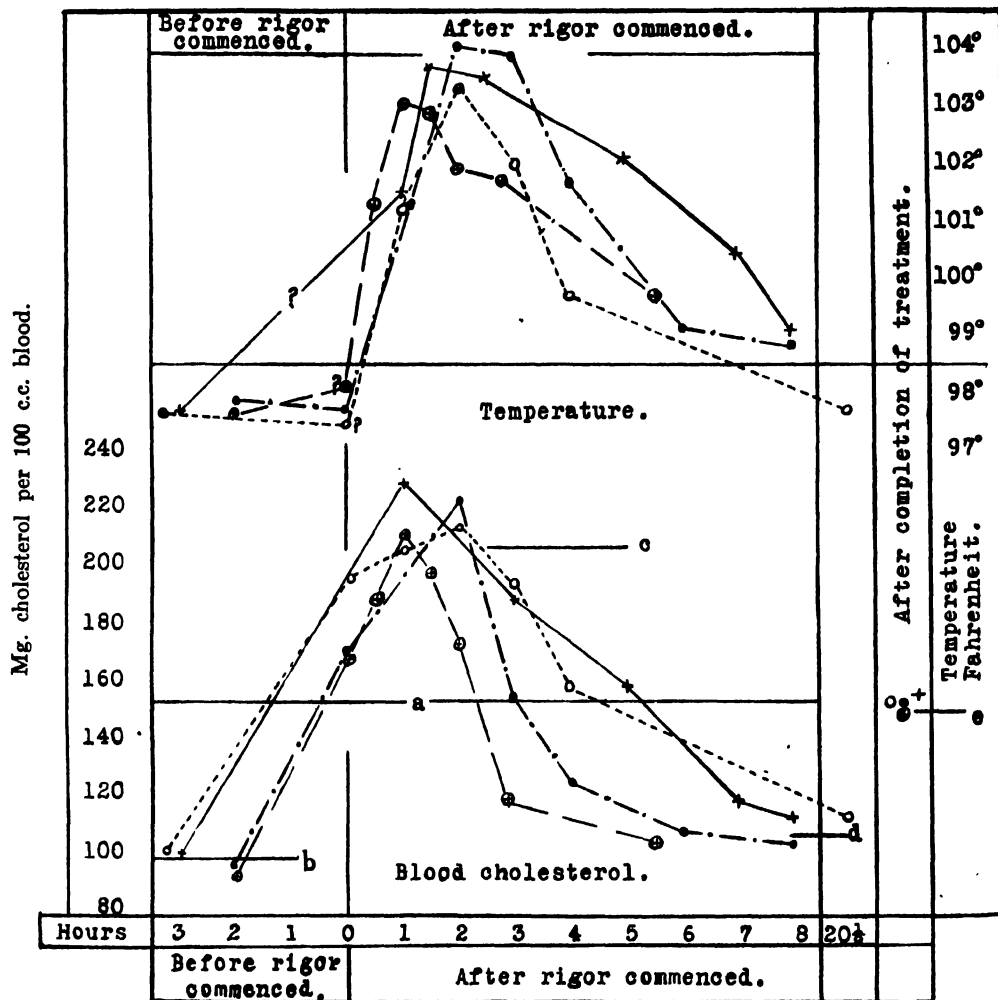
Blood cholesterol level in relation to fever in 25 cases of benign tertian malaria.

The results of the examination of four other benign tertian infections, in which more frequent observations were made during pyrexia, are shown in Text-figure 2. The same rise in blood cholesterol was found. It will be noted that in the post-pyrexial period the decrease in blood cholesterol precedes the fall in temperature. In the case of two malignant tertian infections also the results showed a similar trend.

Besides these, observations were made on 38 benign tertian infections also occurring amongst British patients on whom, however, it was not practicable to make complete records of the cholesterol content of the blood at all stages of the

TEXT-FIGURE 2.

Cholesterol content of blood in relation to the paroxysm in malaria.



Benign tertian malaria. 'a' average of 48 normal cases—155.3. 'b' average of 55 cases before fever—100.31. 'c' average of 39 cases during fever—208.5. 'd' average of 38 cases after fever—108.4. 'e' average of 59 cases after completion of treatment—152.06.

attack. In 27 of these patients cholesterol estimations, carried out during the afebrile period preceding the rigor, gave an average value of 100.15 (maximum 105 : minimum 98). The average figure for 11 patients examined during the febrile attack was 205.1 (maximum 220 : minimum 190). In 10 patients examined after the fever had subsided, the average cholesterol value was 108.5 (maximum 111 : minimum 105), while in 31 cases in which the blood cholesterol was estimated after the completion of specific treatment, the average figure was 155.6 (maximum 180 : minimum 112).

The analysis of the results obtained from benign tertian infections showed that the average cholesterol value for 55 cases examined in the pre-pyrexial stages was 100.31, for 39 cases examined during pyrexia the average figure was 208.5, for 38 patients examined after the fall of fever the average was 108.4, and in 59 cases examined after the completion of treatment the average figure was 152.06.

In every case referred to above the diagnosis was established by the finding of malaria parasites in the peripheral blood.

B. OBSERVATIONS ON MONKEY MALARIA.

Silenus rhesus monkeys (approximately 3 to 5 kg. weight) were used in these experiments. It was not practicable to make estimations in monkeys exactly parallel to those made in human cases as it was difficult to determine clinically the ague stage accurately. Blood was therefore taken in the morning usually about 12 to 16 hours after the last meal. Total plasma cholesterol was estimated in normal, chronic, and treated monkeys. In order to get more complete information, estimations were made on the same animal during normal, incubation, and disease periods.

In order to establish a normal range of cholesterol variations, estimations were made on 40 uninfected animals. The average value was found to be 100 mg. per 100 c.c. plasma (maximum 148 : minimum 56).

In 34 monkeys suffering from chronic *P. knowlesi*, *P. inui*, and *P. cynomolgi* infections, the average cholesterol level was 71 mg. per 100 c.c. plasma.

Cholesterol estimations made during primary *P. knowlesi* infections on 25 monkeys showed very wide variations. The average cholesterol value was 97 mg. per 100 c.c. plasma.

The effect of specific treatment with quinine and plasmoquine on the cholesterol value in 20 monkeys, each having about 20 to 40 thousand parasites per c.c. of blood, showed that from five to seven days after the blood became parasite-free, the cholesterol level reached the normal value.

More complete investigations were carried out on 20 monkeys, and the individual estimations were made as follows :—

- (1) Cholesterol estimations on alternate days for seven to ten days under normal conditions.
- (2) Daily estimations after inoculation with *P. knowlesi*.
- (3) Daily estimations from the time parasites appeared in the peripheral blood till death supervened.

The results of these estimations are given in Table I. The average level of cholesterol in 72 estimations made during the normal period was 103 mg. per 100 c.c. plasma; during the incubation period the average of 32 estimations was 101 mg. and during the period of patent infection the average of 99 estimations was 96 mg. These figures indicate a slight decrease in total blood cholesterol during the period of patent infection as compared with the normal and incubation periods. There was, however, such a wide range of variation between different animals at all periods of observation that no definite

TABLE I.
Showing the amount of cholesterol during the normal, incubation, and disease periods.

Serial number of monkeys.	Number of estimations during normal period.					Number of estimations during incubation period.					Number of estimations during disease period.									
	1	2	3	4	5	1	2	3	4	1	2	3	4	5	6	7	8	9	10	
* 234	115	148	142	129	133	135	118	132	103	111	
* 237	124	137	138	147	132	135	113	145	106	113	56	
246	110	108	104	102	111	102	110	106	107	115	154	105	125	129	87	76	
* 247	112	111	114	110	101	108	110	105	132	100	
* 1A	143	121	98	114	84	64	127	
* 2A	152	165	89	119	107	130	99	169	
3A	139	111	92	136	..	97	99	88	99	81	61	
4A	91	138	129	141	..	113	84	86	68	79	65	
* 6A	83	75	87	63	117	106	53	91	
7A	75	66	61	89	93	..	104	136	
* 8A	64	57	73	56	42	104	
* 9A	95	57	64	40	..	79	113	133	120	
* 11A	103	100	81	74	99	49	84	74	70	60	68	78	
12A	70	51	88	110	76	98	93	85	80	95	91	90	81	71	81	59	102	
* 13A	130	56	79	85	95	72	68	97	132	67	75	80	80	90	
14A	66	66	91	61	100	107	113	89	
* 19A	94	68	94	139	75	82	137	116	
20A	105	84	130	72	82	108	82	64	122	121	
* 21A	94	102	98	161	64	83	79	85	106	
* 22A	138	114	81	98	..	54	54	71	95	67	105	

* Animals which suffered from hæmoglobinuria during the terminal stages of the primary acute attack.

conclusions can be drawn from the results observed. Perhaps the most striking observation made was a considerable and abrupt rise in the cholesterol level shortly before death in those monkeys which passed blackwater (hæmoglobinuria). This phenomenon was not observed in monkeys whose death was not associated with hæmoglobinuria. On the other hand, the latter showed a gradual and continuous fall in the cholesterol level up to the time of death.

Determination of the 'ester' and 'free' fractions of cholesterol on 25 blood samples during the period of maximal parasitæmia showed that the variations are primarily due to a rise in the ester cholesterol. The free cholesterol showed almost a proportionate fall.

LIPOID PHOSPHORUS (LECITHIN) IN RELATION TO CHOLESTEROL IN MALARIA.

Since both cholesterol and lecithin are intermediate steps in fat metabolism, the study of one may be considered incomplete without taking the other into consideration. Furthermore, while there is much that is indefinite in the data available regarding their relationship, it has been claimed that these two substances are essentially antagonistic. It was therefore considered advisable to investigate whether any relationship existed between these two substances in animals infected with malaria.

The lecithin content of plasma and blood was determined by Youngburg's (1930) method. Estimations were first made on a number of healthy monkeys to establish the range of normal variation, and were repeated on *rhesus* monkeys suffering from acute and chronic *P. knowlesi* infections.

Table II gives the results of estimations of lecithin in whole blood and plasma in the normal monkeys as well as in those suffering from acute and chronic infections with *P. knowlesi*. It will be noted that the lecithin level of

TABLE II.

Showing milligrammes of lecithin per 100 c.c. blood or plasma in normal and infected monkeys*.

Blood or plasma lecithin.	Number of observations.	Maximum lecithin value observed.	Minimum lecithin value observed.	Average lecithin value.
Normal—				
Whole blood	24	430.0	272.5	338.0
Plasma	8	342.0	267.5	305.0
Primary <i>P. knowlesi</i> infections—				
Whole blood	12	370.0	250.0	297.5
Plasma	14	345.0	195.0	252.5
Chronic <i>P. knowlesi</i> infections—				
Whole blood	10	297.5	252.5	278.7
Plasma	13	243.0	178.5	239.0

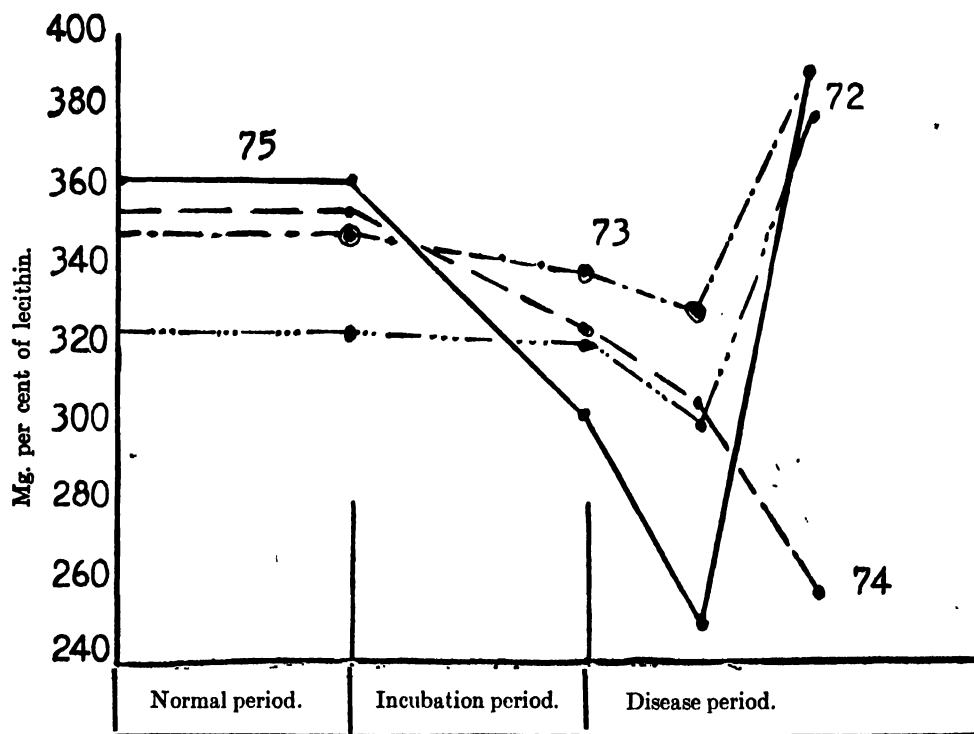
* All estimations on lecithin were done in triplicate and the figures given in this table represent the average value of the three estimations.

monkeys both in acute and chronic infections is considerably lower than normal, and that the fluctuations of the values obtained are very wide during the disease period. It will also be observed that the lecithin level of whole blood was considerably higher than that of plasma in both normal and infected animals.

In order to study more comprehensively the change in the lecithin content of whole blood and plasma with the progress of the disease, observations were carried out on four monkeys during the normal, incubation, and patent infection periods of *P. knowlesi* infections in *S. rhesus* till death supervened.

TEXT-FIGURE 3.

Showing the amount of lecithin during the normal, incubation, and patent infection periods in *P. knowlesi* infections*.



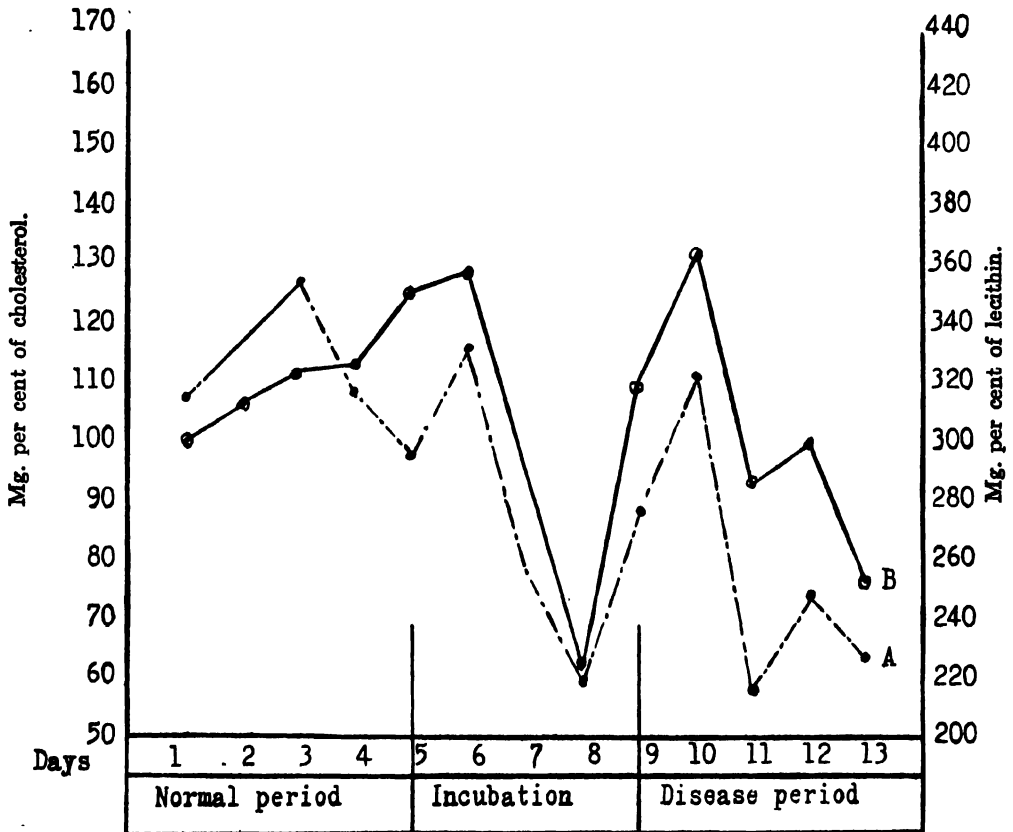
* Monkeys 72, 73 and 75 died with hæmoglobinuria, and monkey 74 without hæmoglobinuria. Lecithin estimations were made on the whole blood of monkeys 74 and 75, and on the plasma of monkeys 72 and 73.

The curves in Text-figure 3 show that there is little change in the lecithin value during the incubation as compared to the normal period, though the general trend of the curves is towards a decrease. During the disease period, however, there is a marked decrease. This decrease was gradual in monkey 74 which did not pass blackwater before death, whereas monkeys 72, 73 and 75 which died with hæmoglobinuria showed an abrupt rise shortly before death.

In two monkeys, parallel estimations of lecithin and cholesterol were made during the normal, incubation, and patent infection periods. Blood was drawn at the same time for both the estimations. The results are given in Text-figures 4 and 5.

TEXT-FIGURE 4.

Whole blood cholesterol and lecithin level of non-haemoglobinuric monkeys during the normal, incubation, and disease periods.



Curves A and B represent the amount of cholesterol and lecithin respectively.

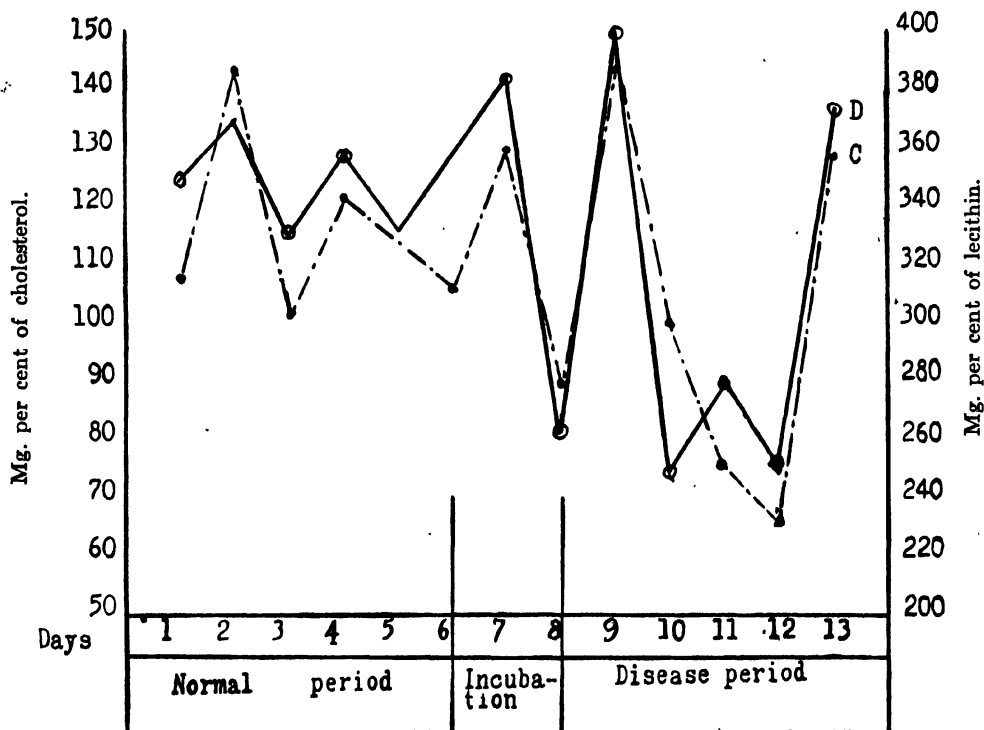
Text-figure 4 shows the variations in the amounts of total cholesterol and lecithin in the whole blood during the normal, incubation, and disease periods in two monkeys that did not die with hæmoglobinuria. The curves give average values of figures obtained from both the animals.

It will be observed that on the 6th day of observation (the day following inoculation with *P. knowlesi*) an increase in both cholesterol and lecithin values was observed followed by a decrease on the 7th and 8th days. When, however, the parasites appeared in the peripheral blood, there was a considerable rise in the value of both these constituents for about two days, followed by a gradual decrease corresponding with the increase in the number

of parasites. It is interesting to note that as the cholesterol value increases the amount of lecithin also increases and with its decrease there is a decrease in lecithin also.

TEXT-FIGURE 5.

Whole blood cholesterol and lecithin level of haemoglobinuric monkeys during the normal, incubation, and disease periods.



Curves C and D represent the amount of cholesterol and lecithin respectively.

In two other monkeys which died with hæmoglobinuria the cholesterol and lecithin estimations were made as in the above case. Text-figure 5 which gives the results of these observations shows that after inoculation on the 6th day there was a rise, and later a decrease, in the value of both cholesterol and lecithin. When the parasites appeared there was an abrupt rise followed by a gradual fall which continued into the terminal stages of infection when the cholesterol and the lecithin values were found to have undergone a sudden and considerable increase, in both cases, before the animals died with hæmoglobinuria.

DISCUSSION OF RESULTS.

The fact that the metabolism of cholesterol and lecithin is still imperfectly understood makes it difficult to interpret the full significance of our findings.

It has been possible as the result of our investigations to obtain a clear picture of the variations in the cholesterol content of the blood at various

stages of the malarial attack. In the actual ague stage of human malaria there is hyper-cholesterolaemia, while in the afebrile stages before and after the paroxysm there is hypo-cholesterolaemia. This variation may explain the discordant results recorded by other workers who have relied on isolated observations without taking into consideration the possibility of changes in cholesterol values at various stages of the attack. The literature on this subject has been reviewed by Campbell (1925) and by Krishnan *et al.* (1936).

It is known that both cholesterol and lecithin are intermediary stages in the metabolism of fat, and that cholesterol is synthesised in the body from both exogenous and endogenous sources. The hyper-cholesterolaemia which occurs during the malarial paroxysm may possibly be explained by an increase in fat metabolism occurring at high body temperatures. If this be so, it seems probable that the cholesterol is synthesised mainly from endogenous sources since the intake of food is usually restricted during the acute attack. It is interesting to note that a similar hyper-cholesterolaemia occurs in anaphylactoid shock, a phenomenon with which the rigor of malaria has many points of similarity (*vide* Sinton and Kehar, 1931). This tends to indicate that the hyper-cholesterolaemia is due to factors unconnected with the presence of the malaria parasites *per se* but may be connected with the febrile and other symptoms resulting from the malarial infection.

With regard to the experiments carried out on monkeys the wide fluctuations observed in the cholesterol content of the blood were not definitely correlated with the paroxysm in the same way as in the human cases, since the occurrence of the paroxysm is difficult to determine clinically and blood slides were not made to determine the time of schizogony.

One of the most significant findings was the striking difference observed in the cholesterol content of the blood in monkeys dying from acute *P. knowlesi* infections without haemoglobinuria and those infected with the same strain of this species of *Plasmodium* which showed haemoglobinuria as a terminal symptom. In the former the cholesterol and lecithin values fell gradually to reach a low level at the time of death (*vide* Text-figure 4). In the latter a similar trend was observed but this showed a sudden and marked increase immediately prior to the occurrence of haemoglobinuria. If haemoglobinuria be precipitated by a reduction in the antihaemolytic factor (cholesterol), one would not expect a sudden rise in this factor immediately before the onset of this condition. On the other hand, this rise may perhaps be a reaction on the part of the body to prevent further red cell destruction. Attempts to counteract a reduction in cholesterol by feeding animals on cholesterol and cholesterol-rich substances from 2 to 54 hours prior to the time at which haemoglobinuria might be expected to occur were not attended with success. Neither lanolin by the oral route nor cholesterol in olive oil (5 to 300 mg. cholesterol) administered intramuscularly in spaced doses of 5 to 50 mg. prevented the occurrence of haemoglobinuria in 21 monkeys.

Our results show that the cholesterol and lecithin contents of the blood in malaria vary directly. A rise or fall in the one is associated with a corresponding rise or fall in the other (*vide* Text-figures 4 and 5). The fact that an increase of lecithin did not precede an increase of cholesterol is not in accordance with the findings of McQuarrie and Stresser (1932), who showed that in fat metabolism the increase in lecithin precedes that in cholesterol by a lengthy

interval. If the occurrence of hæmoglobinuria were attributable to an increase of lecithin in the absence of a corresponding increase of cholesterol it would not be expected that the curves of the two substances should show such a close parallelism throughout the malarial infection.

SUMMARY.

Observations on cholesterol changes in both human and monkey malaria and on lecithin changes in monkey malaria are presented in this paper.

1. *Human malaria.*

It has been found that :—

- (i) There is hyper-cholesterolæmia during the rigor stage, and hypo-cholesterolæmia during the afebrile stages before and after the paroxysm as well as in chronic infections.
- (ii) There is a high correlation between the cholesterol content and the oral temperature at the paroxysm stage. After the paroxysm is over the fall in the amount of cholesterol precedes the fall of temperature.
- (iii) With the commencement of specific treatment (quinine and plasmoquine) there is a gradual rise in the cholesterol content of the blood which reaches the normal level after about 15 days' treatment.

2. *Monkey malaria.*

- (i) The average of cholesterol estimations on 20 monkeys during the normal, incubation, and disease periods shows that practically no change occurs during the incubation period compared to the normal, but a slight hypo-cholesterolæmia is observed during the primary acute attack.
- (ii) The variations in the quantity of total cholesterol are due to a rise in the ester cholesterol and a fall in the free cholesterol.
- (iii) Monkeys which die with hæmoglobinuria show an abrupt increase in the cholesterol content of the plasma of the peripheral blood just before death, while the estimations made on others that do not die with hæmoglobinuria show a gradual fall. This is also true of lecithin.
- (iv) There seems to be a direct correlation between the amount of cholesterol and lecithin present in the body in both healthy and infected monkeys.
- (v) Feeding of cholesterol-rich substances or intramuscular injections of cholesterol did not protect monkeys from the onset of hæmoglobinuria.

ACKNOWLEDGMENTS.

In conclusion I wish to express my gratitude to Lieut.-Col. J. A. Sinton, M.D., D.Sc., V.C., O.B.E., I.M.S., formerly Director, Malaria Survey of India, for his suggestions, help, encouragement and criticism in carrying out this investigation and to Major H. W. Mulligan, M.D., D.Sc., I.M.S., for much help in the preparation of this paper for publication. I am also thankful to Sub-Assistant Surgeon Harbhagwan, D.T.M., I.M.D., for assistance.

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SEDIMENTATION RATE OF ERYTHROCYTES IN HUMAN AND MONKEY MALARIA.

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INTRODUCTION.

SINCE Fahraeus (1918) revived interest in the rate with which the red blood cells sediment in different pathological conditions, considerable attention has been paid to its value in the prognosis and diagnosis of various diseases.

Although most workers agree that the erythrocyte sedimentation rate is increased in febrile conditions, no extensive experimental observations appear to have been made on the sedimentation rate in monkey malaria, and previous observations on human malaria are meagre and inconclusive. In view of the importance of the sedimentation rate in other diseases, an investigation was commenced in 1932 to determine the sedimentation rate during the various stages of the malarial attack.

METHODS AND MATERIAL USED.

These observations were made by the micro-method (Trail and Stone, 1929) with the Hellige Sedimentassometer. This method has the advantage that very small amounts of blood are required, permitting frequent observations over long periods on the same animal. The animals were bled after a starvation period of about 16 hours to eliminate the influence of food intake, since it has been pointed out by Balachowsky (1925) that the sedimentation rate is increased immediately after meals.

Readings were taken at intervals of 10 minutes and were continued over an observation period of two hours. The length of the clear fluid column

above the sedimented red cells was read in mm. with the help of a hand lens. This gave the result directly in mm. per cent. The observations were taken at room temperature since Newham (1926) demonstrated that slight variations in temperature did not influence the rate of sedimentation.

The investigations carried out on monkey and human malaria may be classified as follows :—

A. OBSERVATIONS ON MONKEY MALARIA.

All monkeys used were young specimens of *S. rhesus*. Observations were made on infections with *P. knowlesi*, *P. cynomolgi*, and *P. inui* and may be summarised as follows :—

1. Sedimentation rate in uninfected monkeys.
 - (a) Single observations on a series of normal monkeys.
 - (b) Repeated observations on individual monkeys carried out daily for several days.
2. Sedimentation rate in infected monkeys.
 - (a) During normal, incubation, and disease periods in untreated *P. knowlesi* infections.
 - (b) In treated *P. knowlesi* infections.
 - (c) In chronic infections with *P. cynomolgi* and with *P. inui*.

B. OBSERVATIONS ON HUMAN MALARIA.

1. Sedimentation rate in healthy persons.
2. Sedimentation rate in infected persons.
 - (a) During various phases of infections with *P. vivax*, *P. falciparum* and mixed infections, to whom no specific treatment had been administered.
 - (b) During similar phases of infections in persons who had received treatment with quinine.

RESULTS OF EXPERIMENTS.

A. MONKEY MALARIA.

1. SEDIMENTATION RATE IN UNINFECTED MONKEYS.

We have not been able to find any record of the sedimentation rate of erythrocytes in monkeys. It was therefore necessary to establish the normal range of variation in healthy animals. This was carried out in—

- (a) a series of 8 healthy monkeys on each of which only a single sedimentation estimation was made, and in which the range of individual variation was found to lie between 1.5 and 3.5 mm. per cent, and in
- (b) another series of 4 healthy monkeys in which daily estimations were made on each for periods of 10 to 15 days. No deviation from the range of variation stated above was observed, in spite of successive daily bleedings.

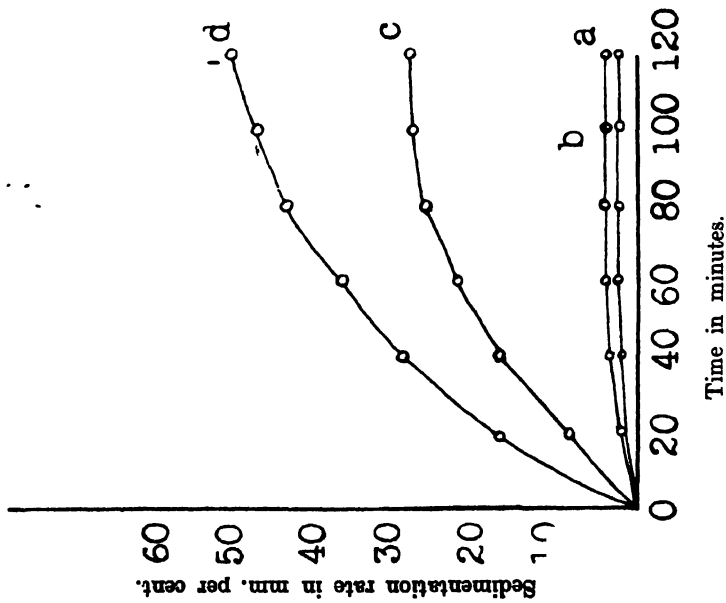
2. SEDIMENTATION RATE IN INFECTED MONKEYS.

- (a) *Sedimentation estimations during untreated infections with P. knowlesi.*

Plasmodium knowlesi was used in these experiments because it produces symptoms of great severity in a susceptible host such as *S. rhesus*. The infection almost invariably proves fatal, if untreated. Observations were made

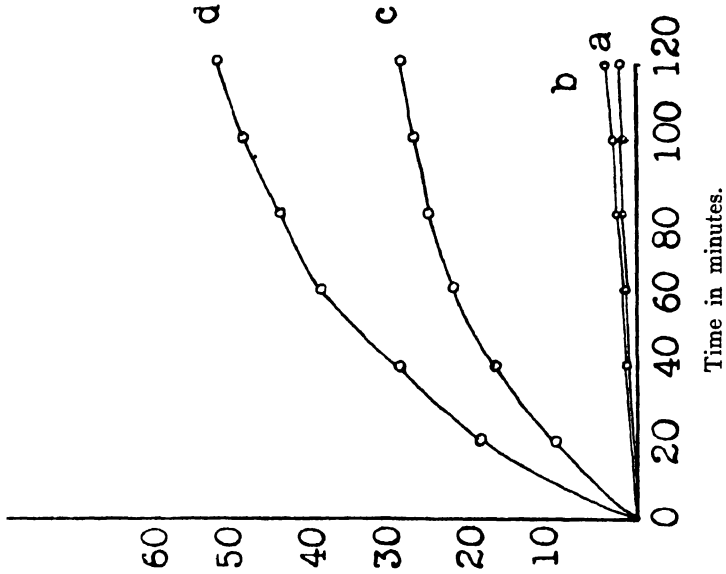
GRAPH 1.
Showing the sedimentation rate during the normal, incubation, and acute disease periods in *P. knowlesi* infections in *S. rhesus*.

Fig. 1.



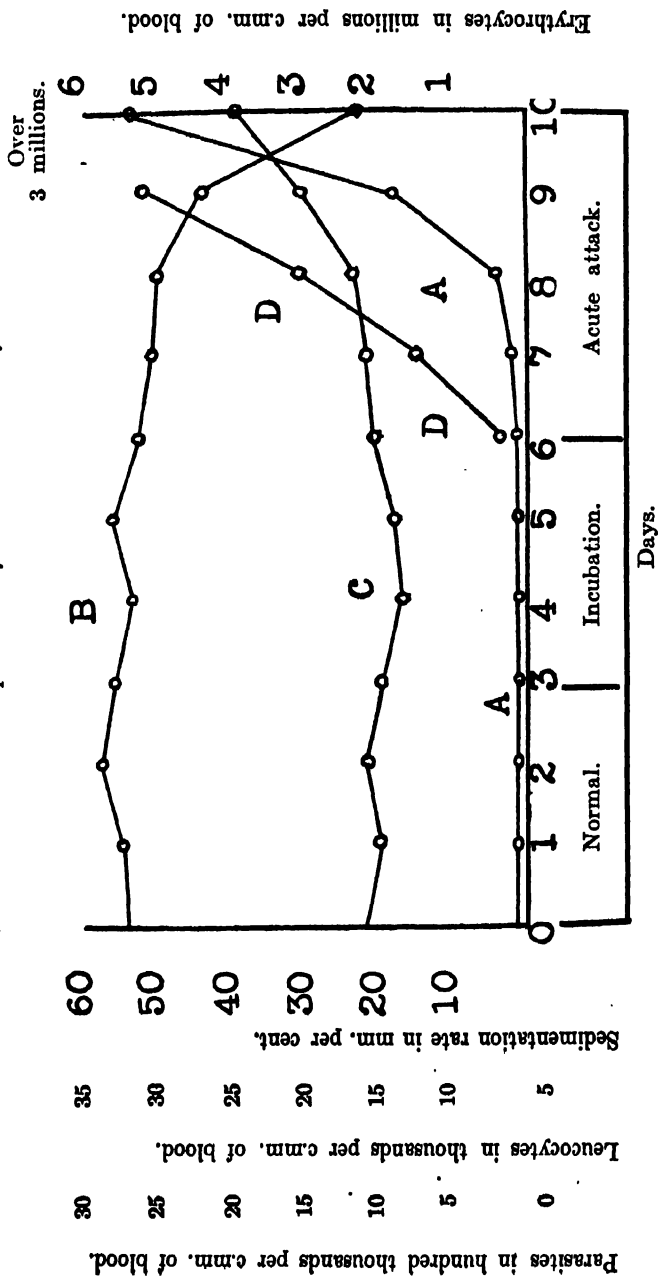
Figs. 1 and 2 represent the sedimentation rate in (a) the normal animal, (b) during the incubation period, (c) during the acute primary attack, and (d) just before death.

Fig. 2.



GRAPH 2.

Showing the sedimentation rate (A), the total erythrocyte count (B), the total leucocyte count (C), and the parasite count (D) during the normal, incubation, and acute disease periods of *P. knowlesi* infections in *S. rhesus*.



on 5 monkeys. The sedimentation rate was observed in the normal animal on alternate days for about one week before inoculation with *P. knowlesi*, and the sedimentation rate was subsequently recorded during the incubation and the disease periods till death supervened. In some cases a control monkey was also used, the blood being drawn from the control animal at the same time and under the same conditions as for the infected monkeys.

Since the results obtained were almost identical in all the infected animals, it was not considered necessary to sacrifice additional monkeys. Experimental data for only two of these monkeys are given in Graph 1, figs. 1 and 2, to economise space.

The results illustrated in Graph 1, figs. 1 and 2, indicate that during the incubation period the sedimentation rate is but little affected. There is, however, a marked increase in this rate during the disease period, particularly when the parasitæmia is very intense and death is imminent.

In a further series of 5 monkeys, similar to those described above, observations on the sedimentation rate were repeated and at the same time parasite counts were made by Sinton's (1924) fowl cell method. Total erythrocyte and leucocyte counts were also made. The results in all 5 animals were similar and those of a representative case are given in Graph 2.

Graph 2 indicates that as the parasite count increases the sedimentation rate and the leucocyte count also increase, while a corresponding decrease is observed in the number of erythrocytes.

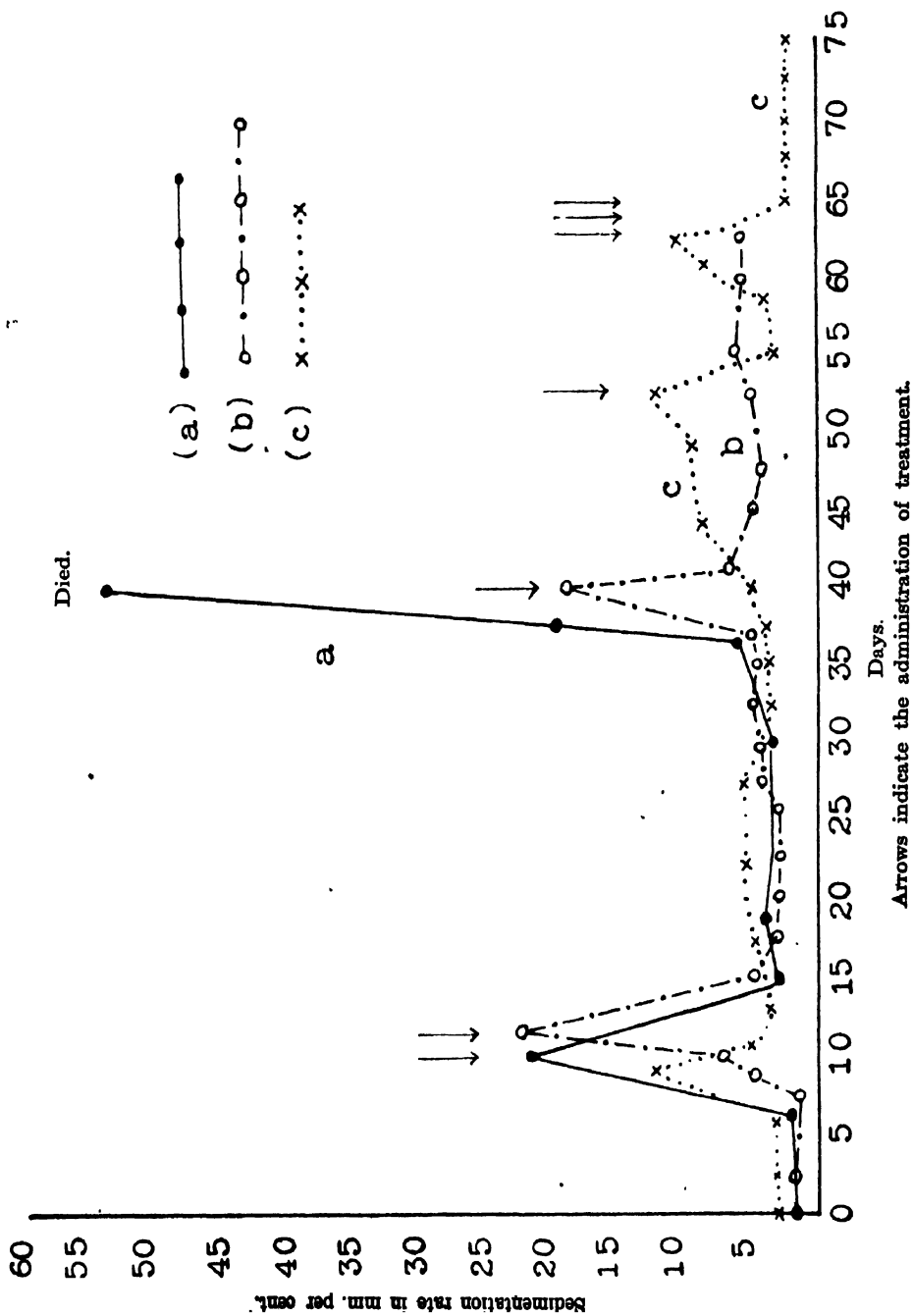
(b) *Sedimentation rate in treated P. knowlesi infections.*

Observations were made on 6 monkeys in the same manner as described above except that treatment was given to modify the course of the infections. In each case, 0.1 gm. atebirin was administered intramuscularly as soon as the parasite count reached from 15,000 to 30,000 per c.mm. of blood. This was found sufficient to check the progress of the disease and the infections usually remained at a low level for a few days after which the parasites again began to increase in numbers. When the latter tendency was observed sedimentation estimations were made as under—

- (i) No further treatment was given to two of the monkeys and the sedimentation rate was observed at intervals until the parasitic infections became very severe and death supervened (*vide* Graph 3, a).
- (ii) Two other monkeys received just sufficient additional atebirin treatment to keep the infection at the sub-clinical level and sedimentation estimations were made at frequent intervals over a period of nine weeks (*vide* Graph 3, b).
- (iii) The two remaining monkeys received treatment as in (ii) above, and after the lapse of about seven weeks sufficient treatment was given to cure the infection, at least temporarily. Sedimentation estimations were made at frequent intervals throughout the whole period of observation (*vide* Graph 3, c).

The results observed in both the monkeys in each of the above categories were in close agreement. These are illustrated in Graph 3 which gives the data on one monkey from each of the above categories. It will be observed

Showing the effect of incomplete (a and b) and complete (c) treatment on the sedimentation rate in *P. knowlesi* infections in *S. rhesus*.



that, in treated infections also, there is a marked increase in the sedimentation rate as the parasite count increases but that when sufficient treatment is given to check or eliminate the parasites there is a corresponding decrease in the rate of sedimentation.

Estimations were also made on a series of monkeys suffering from chronic or latent infections with *P. knowlesi*. In 56 such animals the average sedimentation rate was 4.7 mm. per cent. In 8 other monkeys suffering from clinical relapses, the average sedimentation rate was 38.1 mm. per cent. In those animals in which a complete cure appeared to have been effected, the sedimentation rate was observed to fall within normal limits.

(c) *Sedimentation rate in chronic infections with P. cynomolgi and P. inui.*

Infection of *S. rhesus* with either *P. cynomolgi* or *P. inui* results in a malarial infection of moderate severity from which recovery is invariably spontaneous and in which there is a subsequent prolonged phase of chronic or latent infection.

Thirty sedimentation estimations were made on 10 monkeys suffering from chronic *P. cynomolgi* infections and the average sedimentation rate was found to be 10 mm. per cent. Similarly 27 observations made on 5 monkeys suffering from chronic infection with *P. inui* gave an average sedimentation rate of 5.4 mm. per cent.

There is considerable evidence to show, therefore, that in chronic or latent infections with *P. knowlesi*, *P. cynomolgi* or *P. inui*, the sedimentation rate tends to be slightly increased but that when cure has been effected the sedimentation rate falls within normal limits.

During the course of these experiments, one monkey infected with *P. knowlesi* proved resistant to infection, and although parasites appeared in scanty numbers from the 4th to the 17th day after inoculation, recovery was spontaneous. This is a very exceptional occurrence with *P. knowlesi* infection in *S. rhesus*. During the period when parasites were present in the blood, the average of several sedimentation estimations was 3.5 mm. per cent. At a later date, when no trace of infection could be discovered, the sedimentation rate was observed to be within normal limits.

(d) *Sedimentation rate and surface tension of serum.*

During an investigation of possible changes in the surface tension of blood serum from monkeys infected with *P. knowlesi*, the opportunity was taken to observe whether any relationship existed between the sedimentation rate and the surface tension of the serum. These observations were made on 6 normal and 6 heavily parasitised monkeys and the results are given in Table I.

Table I shows that while the average surface tension and sedimentation rate in normal monkeys is 82.4 dynes and 2.3 mm. per cent respectively, the corresponding figures for heavily parasitised monkeys are 80.4 dynes and 62.5 mm. per cent. There is, therefore, a decrease in the surface tension of the serum and a marked increase in the sedimentation rate of erythrocytes in severe infections with *P. knowlesi* in *S. rhesus*.

TABLE I.

Showing the sedimentation rate and surface tension in normal and in heavily parasitised monkeys.

Serial number of monkeys.		SEDIMENTATION TIME IN MINUTES.								Surface tension.
		10	20	30	40	60	80	100	120	
1	Normal	0.05	0.05	0.1	0.15	0.2	0.25	84.2
2	"	..	0.05	0.1	0.15	0.15	0.2	0.25	0.25	83.5
3	"	..	0.05	0.1	0.15	0.15	0.2	0.2	0.25	83.2
4	"	..	0.1	0.1	0.15	0.2	0.2	0.25	0.25	81.8
5	"	..	0.05	0.05	0.05	0.1	0.15	0.15	0.2	82.9
6	"	..	0.05	0.05	0.05	0.1	0.2	0.2	0.2	83.7
Average	"	82.4
51	<i>P. knowlesi</i>	..	2.9	3.2	3.6	3.8	4.5	4.8	5.5	80.5
52	"	0.4	1.4	2.0	2.9	3.8	4.0	4.5	4.8	81.2
54	"	1.8	3.5	5.1	5.7	6.3	6.6	6.6	6.7	80.0
59	"	1.5	2.4	3.2	4.5	5.8	6.2	6.8	7.0	80.2
61	"	0.3	0.7	1.2	2.4	2.9	3.7	4.2	4.5	82.0
65	"	1.5	3.2	5.7	6.5	7.5	8.3	8.5	9.0	79.5
Average	"	80.4

B. OBSERVATIONS ON HUMAN MALARIA.

1. NORMAL SEDIMENTATION RATES.

Sedimentation estimations were made on 11 healthy members of the laboratory staff living in a malaria-free locality. The average of these estimations was 6.4 mm. per cent, a figure which is appreciably higher than that obtained in the case of normal monkeys.

2. SEDIMENTATION RATES IN HUMAN MALARIA CASES.

Through the courtesy of Major H. W. Mulligan, I.M.S., we are including in this paper sedimentation observations made by him on human malaria cases at the Indian Military Hospital, Rawalpindi, in 1931, using the same technique as that which has been described above. Observations were made on 48 cases of which 38 were infections with *P. falciparum*, 8 with *P. vivax*, and 2 with mixed *P. falciparum* and *P. vivax* infections. In 40 of these cases parasites were present in the peripheral blood at the time of sedimentation estimation, while in the remaining 8 cases parasites had disappeared from the blood following the exhibition of specific treatment. Brief clinical notes on each of the above cases are given in Table II, and the sedimentation rates of patients at various stages of the disease are shown graphically in Graphs 4 and 5.

Graph 4 (figs. 1 to 8) indicates the sedimentation rate in infections with *P. falciparum*, and Graph 5 (figs. 1 and 2) the sedimentation rate in *P. vivax* infections.

TABLE II.

Notes on human malaria cases on which sedimentation estimations were made.

Serial number.	CLINICAL NOTES.				Quinine treatment given.
	Temperature, °F.	Parasites *.	Spleen size †.	Stage of infection.	
(a) Patients infected with <i>P. falciparum</i> .					
1	Afebrile	Rings +	3 F	After paroxysm	2 × 10 grains
2	99.0	Rings +	P	"	"
3	99.5	Rings +	1 F	"	"
4	Afebrile	Rings +	—	"	"
5	"	Rings + + +	—	Algid case	Nil
6	"	Rings +	—	Between paroxysms	"
7	"	Rings + +	P	"	"
8	"	Rings +	1 F	"	"
9	"	Rings + +	1 F	"	"
10	99.2	Rings +	—	"	"
11	99.6	Rings + +	3 F	"	"
12	Afebrile	Rings + +	P	"	"
13	"	Rings +	1 F	Before paroxysm	"
14	"	Rings +	P	"	"
15	"	Rings +	P	"	"
16	101.5	Rings +	—	"	"
17	Afebrile	Rings +	1 F	"	"
18	104.0	Rings + + +	P	During paroxysm	"
19	Afebrile	Rings +	P	Between paroxysms	"
20	"	Rings +	—	"	"
21	"	Rings +	—	"	"
22	"	Rings + +	P	"	"
23	"	Rings + + +	—	"	"
24	"	Rings + +	P	"	"
25	"	Rings +	—	"	"
26	"	Rings +	4 F	"	"
27	"	Rings + +	2 F	"	"
28	98.6	Rings +	P	"	"
29	101.5	Rings +	—	During paroxysm	"
30	Afebrile	Rings +	2 F	Between paroxysms	"
31	"	Nil	—	Post-febrile	3 × 10 grains daily.
32	"	"	2 F	"	"
33	"	"	2 F	"	"
34	"	"	2 F	"	"
35	"	"	3 F	"	"
36	"	"	P	"	"
37	"	"	P	"	"
38	"	"	P	"	"

* A rough indication of the number of parasites present at the time of estimation of the sedimentation rate is given by the signs +, ++, +++, etc.

† P = palpable; other rough splenic measurements are indicated in finger-breadths, e.g., 1F = one finger-breadth below the costal margin. Where no figure is given the spleen was not palpable.

TABLE II—concl'd.

Serial number.	CLINICAL NOTES.				Quinine treatment given.
	Temperature, °F.	Parasites *.	Spleen size †.	Stage of infection.	
(b) Patients infected with <i>P. vivax</i> .					
1	Afebrile	Rings and gametocytes.	—	Between paroxysms	2 × 10 grains
2	"	"	—	"	"
3	"	Trophozoites	P	"	"
4	99.8	Rings ++	—	During " paroxysm	Nil
5	Afebrile	Rings +	—	Between paroxysms	"
6	"	Rings +	—	"	"
7	"	Rings +	—	"	"
8	"	Rings and trophozoites.	P	"	"
(c) Patients infected with mixed <i>P. vivax</i> and <i>P. falciparum</i> .					
1	Afebrile	Rings, etc.	—	Between paroxysms	Nil
2	"	"	—	"	"

* A rough indication of the number of parasites present at the time of estimation of the sedimentation rate is given by the signs +, ++, +++, etc.

† P = palpable; other rough splenic measurements are indicated in finger-breadths, e.g., 1F = one finger-breadth below the costal margin. Where no figure is given the spleen was not palpable.

It will be observed from these figures that all cases of human malaria showed an increase in the sedimentation rate. It has not been possible to correlate this with the species of parasite present or with the clinical findings in each case. Thus, while the sedimentation rate was high in some cases showing enlargement of the spleen, it was relatively low in other such cases, while other cases with no palpable enlargement of the spleen showed a high sedimentation rate. Similarly there was no direct correlation between body temperature and sedimentation rate. From the data available there was no evidence that the sedimentation rate in the *P. falciparum* cases was higher than in those suffering from *P. vivax*. It is possible that there may be some relationship between the degree of parasitæmia and the sedimentation rate. Thus in cases 5, 8 and 23, where the parasitic infection was exceptionally high, the sedimentation rate was also increased to a very high degree.

DISCUSSION OF RESULTS.

The factors responsible for variations in the sedimentation rate are not clearly understood but it has been suggested by other workers that this variation may be related to (a) the degree of anæmia present, (b) the protein content of the serum, and (c) certain physico-chemical alterations in the blood.

(a) In our work on monkeys it was observed that a relationship existed between the total erythrocyte count and the sedimentation rate. At the height of the malarial infection when the erythrocyte count fell to about 2 to 3 million per mm. from a normal figure of about 6 million, the sedimentation

GRAPH 4.
Showing the sedimentation rate* in acute infections with *P. falciparum*.

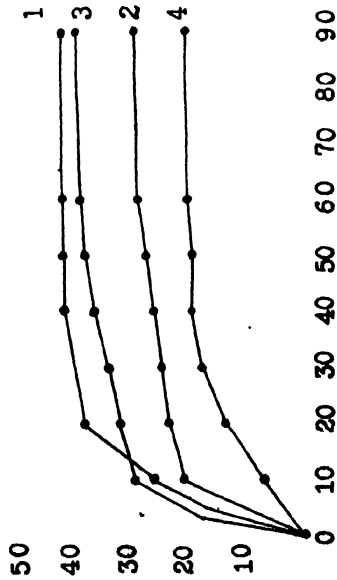


Fig. 1.

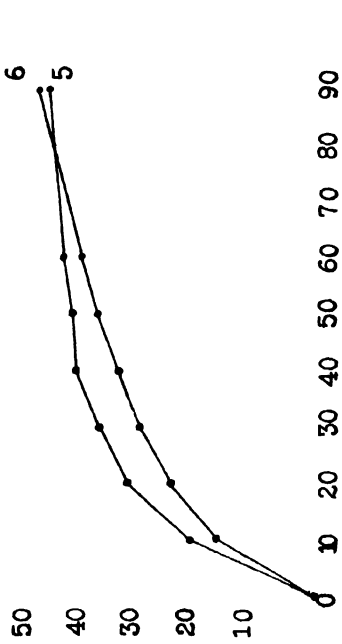


Fig. 2.

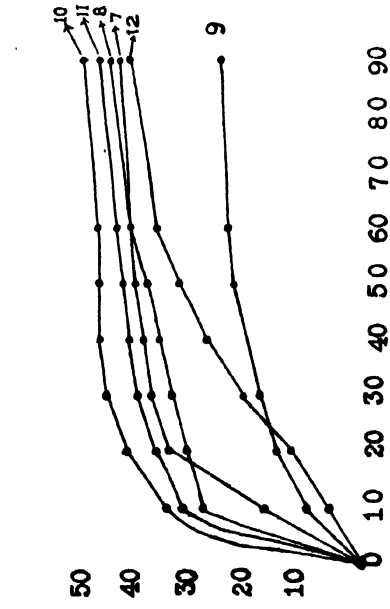


Fig. 3.

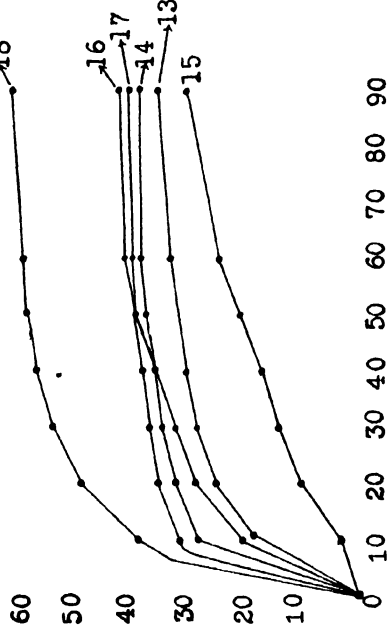


Fig. 4.

GRAPH 4—concl'd.

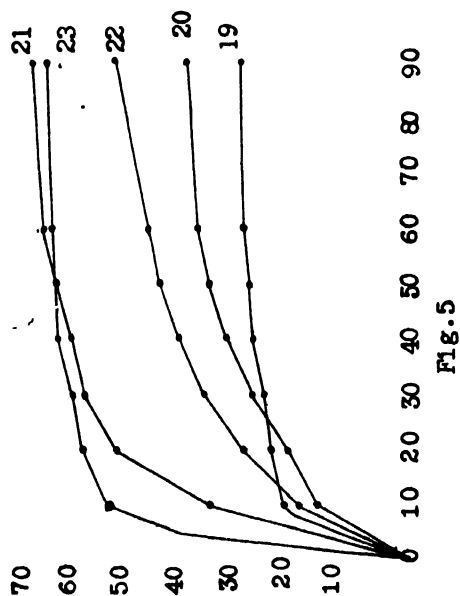


Fig. 5

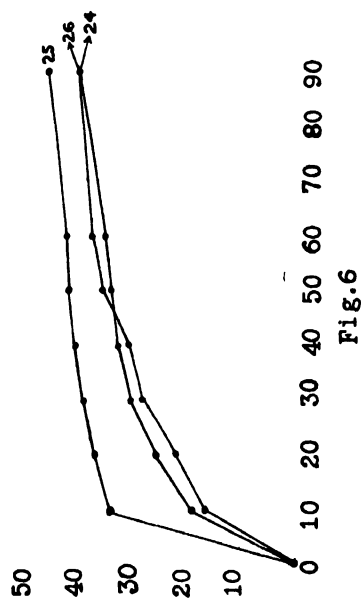


Fig. 6

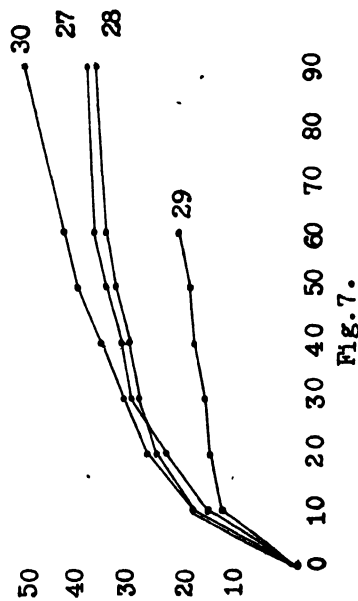


Fig. 7.

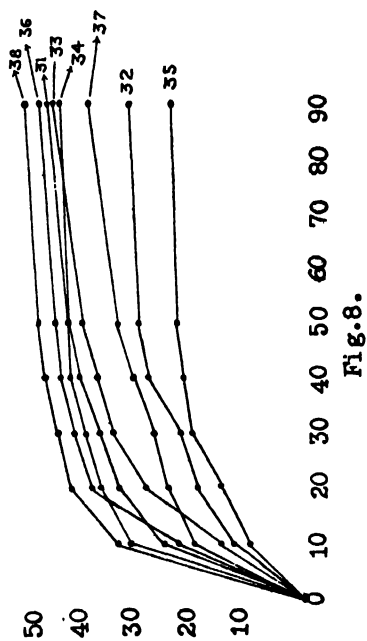


Fig. 8.

* In all figures the abscissæ represent time in minutes and the ordinates the sedimentation rate in mm. per cent.

GRAPH 5.
Showing the sedimentation rate* in acute infections in *P. vivax*.

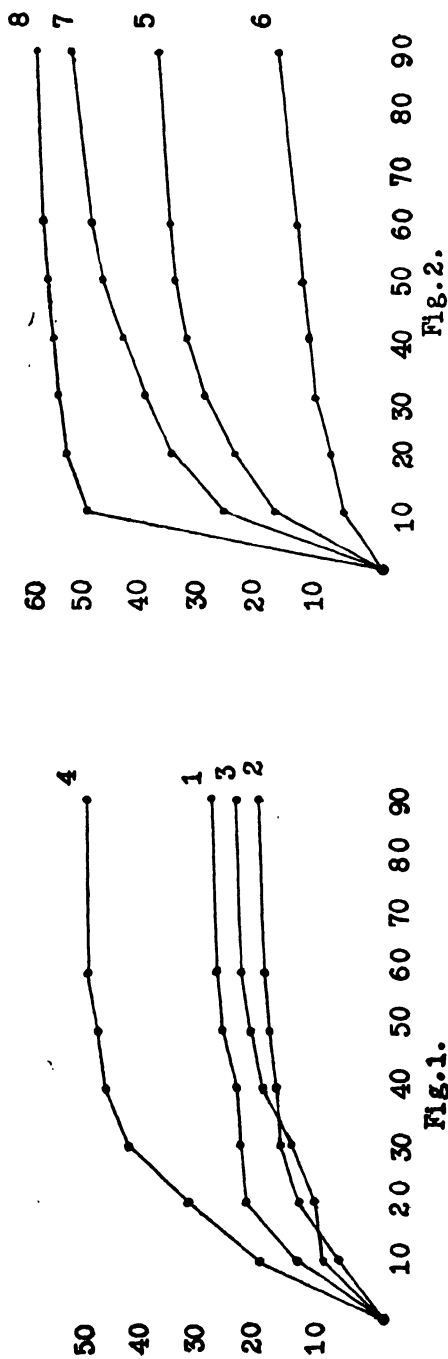


Fig.1.

* See footnote to Graph 4.

Fig.2.

rate rose from a normal figure of 2.5 mm. per cent to about 56 mm. per cent during the disease period. During the incubation period, however, although the erythrocytes showed a slight decrease, the sedimentation rate did not show any significant variations. It must be remembered in this connection that with the decrease in the total erythrocyte count at the height of infection, there is concomitantly a very great increase in the number of parasites present in the peripheral blood, and this may perhaps be responsible, at least in part, for the marked increase in the sedimentation rate.

(b) Several workers have suggested that alteration in the globulin-albumin ratio of the serum with an increase in the globulin content is responsible for an increase in the rate of sedimentation (Schmitz and Schmitz, 1926; Sachs, 1919; Solomon, 1924; Fischel, 1924). Chopra, Mukherjee and Sen (1934) studied the serum protein changes in human malaria and found that all the protein fractions deviated from normal, e.g., albumin diminished considerably, euglobulin increased to a certain extent, while pseudo-globulin showed but slight departures from normal. Ghosh and Sinton (1935) also reported a rise in globulin in malaria. Kehar (1936) found that in monkeys infected with *P. knowlesi*, as the serum globulin increased in acute infections, there was a concurrent and almost proportional increase in the sedimentation rate.

(c) Sinton, Orr and Ahmad (1928) reported a decrease in the surface tension of the serum during the rigor stage in human malaria. Kehar (1936) found that there was a considerable decrease in the surface tension of the serum during the active disease period in monkey malaria (*P. knowlesi*). Further data in confirmation of this observation have been given above. This author also found an increase in refractive index and a decrease in pH when the parasite infestation was very heavy. It is possible that the changes observed in the sedimentation rate in malaria may be attributable to one or more of the above factors or to the cumulative effects of a number of them.

It must be admitted that while there is evidence of some correlation between the sedimentation rate in malaria and the various other changes in the blood which have been mentioned above, there is as yet no proof of the cause of the increased sedimentation rate and it is possible that it may be dependent upon other biochemical and biophysical factors affecting either the erythrocytes, the blood serum, or both.

Whatever the cause of the increased rate of sedimentation the evidence adduced in this paper clearly indicates that this is a consistent change. So far as human malaria is concerned, our results are in accord with those previously recorded (Newham, 1926).

SUMMARY.

Observations on the sedimentation rate of erythrocytes in human and monkey malarial infections as well as in healthy subjects are presented. The following conclusions appear to be justified from the data given:—

A. *Monkey malaria.*

- (1) The average sedimentation rate in healthy *rhesus* monkeys is 2.0 mm. per cent.

- (2) Observations made on monkey malarial infections during the normal, the incubation, and the disease periods indicate that there is a gradual increase in the sedimentation rate after parasites appear in the peripheral blood, and an abrupt and marked increase when parasitæmia is maximal.
- (3) During chronic infections the sedimentation rate is slightly higher than normal, and when relapses occur there is a further abrupt rise in the sedimentation rate.
- (4) Administration of specific treatment has a pronounced effect in lowering the sedimentation rate. When sufficient treatment is given to effect a temporary reduction in the number of parasites, the sedimentation values remain a little higher than normal, while in cases where sufficient treatment is given to effect what was believed to be a complete cure, the sedimentation rate soon reaches the normal level.

B. Human malaria.

- (1) The average sedimentation rate in normal human subjects was found to be 6.4 mm. per cent.
- (2) The available evidence indicates that the changes in the sedimentation rate in human malaria are very similar to those observed in monkey malaria. It was found that there is a considerable increase in the sedimentation rate during the acute phases of the infection. From the data available there is no evidence that the rate of sedimentation differs appreciably in infections with *P. vivax* and *P. falciparum*.
- (3) Similarly, the sedimentation rate in human malaria could not be definitely correlated with such clinical findings as body temperature and the degree of splenic enlargement.

The cause of the increased rate of sedimentation which appears to be a constant change during the active phases of both human and monkey malaria is not definitely understood. It seems probable that these changes may be dependent upon various physico-chemical alterations affecting the solid and fluid elements of the blood.

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THE RELATIVE PREVALENCE OF ANOPHELINES IN HOUSES AND CATTLE-SHEDS IN DELTAIC BENGAL.

BY

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[11th January, 1937.]

INTRODUCTION.

VERY little is on record about the daytime resting places of the Indian anophelines. With the intention of finding out to what extent the anopheline species are partial to animal quarters an extensive search was made for adults both in cattle-sheds and dwelling rooms in several villages lying on either bank of the River Hooghly extending downstream from Calcutta to Falta and Uluberia. This investigation was carried out during the period August 1933 to July 1934.

The results herein detailed are based on 1,133 catches from cattle-sheds, and 432 catches from dwelling rooms occupied mostly by poor class people who live in huts with thatched roofs and mud walls in the riparian villages. Altogether 15,453 anophelines of various species (12,156 from cattle-sheds and 3,297 from rooms) were encountered in the catches during the period. The number of rooms examined is rather low, as in many places the occupants refused to allow the mosquito catchers to enter their rooms and, secondly, unlike the cattle-sheds, all the houses did not show the presence of anophelines. The mosquitoes were collected usually in the forenoon.

OBSERVATIONS.

The species represented in these systematic catches, almost every day, were *A. subpictus* Grassi, *A. vagus* Don., *A. hyrcanus* var. *nigerrimus* Giles, *A. barbirostris* Van der Wulp, *A. sundaicus* Roden., *A. varuna* Iyengar, *A. aconitus* Don., *A. annularis* Van der Wulp, *A. ramsayi* Covell, *A. philippinensis* Lud., and *A. culicifacies* Giles. In addition to these a few specimens of *A. pallidus* Theo. and *A. tessellatus* Theo. were occasionally encountered. The proportionate distribution of the various species in standardised samples of ten catches during the twelve months, August 1933 to July 1934, is shown in Tables I and II. The relative prevalence of the species in the sheds and rooms is also expressed in terms of percentage side by side with the numerical frequency of the various species.

TABLE I.
Incidence of anophelines in human habitations.
(Per ten standardised catches.)

	1933.					1934.							Entire period.
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	
<i>A. barbirostris</i>	2 5.5	..	7 4.8	8 17.0	3 2.4	30 27.7	7 12.7	5 7.4	62 6.2
<i>A. hyrcanus</i> ..	2 4.5	5 13.9	..	36 24.6	5 10.6	3 2.4	23 21.3	5 9.1	3 4.4	1 1.0	83 8.4
<i>A. subpictus</i> ..	9 20.4	2 1.3	2 4.2	3 2.4	..	5 9.1	16 23.8	39 37.1	50 64.1	45 45.4	171 17.3
<i>A. vagus</i> ..	22 50.0	10 27.7	60 73.1	31 21.2	11 23.4	7 5.7	27 25.0	20 36.3	10 14.6	15 14.2	15 19.2	35 35.3	263 26.6
<i>A. sundanicus</i> ..	5 11.3	2 5.5	4 4.8	8 5.5	4 3.6	48 45.7	12 15.3	14 14.1	97 9.8
<i>A. annularis</i> ..	1 2.2	16 44.4	15 18.3	49 33.5	14 29.8	103 84.4	17 15.7	14 25.4	29 43.3	3 2.8	..	2 2.0	263 26.4
<i>A. philippinensis</i>	1 2.7	1 1.2	3 2.0	2 4.2	7 0.7
<i>A. ramsayi</i> ..	4 9.1	3 2.0	2 4.2	1 1.5	1 1.0	11 1.1
<i>A. varuna</i> ..	1 2.2	..	2 2.4	5 3.4	1 2.1	2 3.6	2 2.9	13 1.3
<i>A. aconitus</i>	2 1.4	2 4.2	3 2.4	7 6.4	2 3.6	1 1.2	1 1.0	18 1.8
<i>A. culicifacies</i>	1 1.5	1 0.1

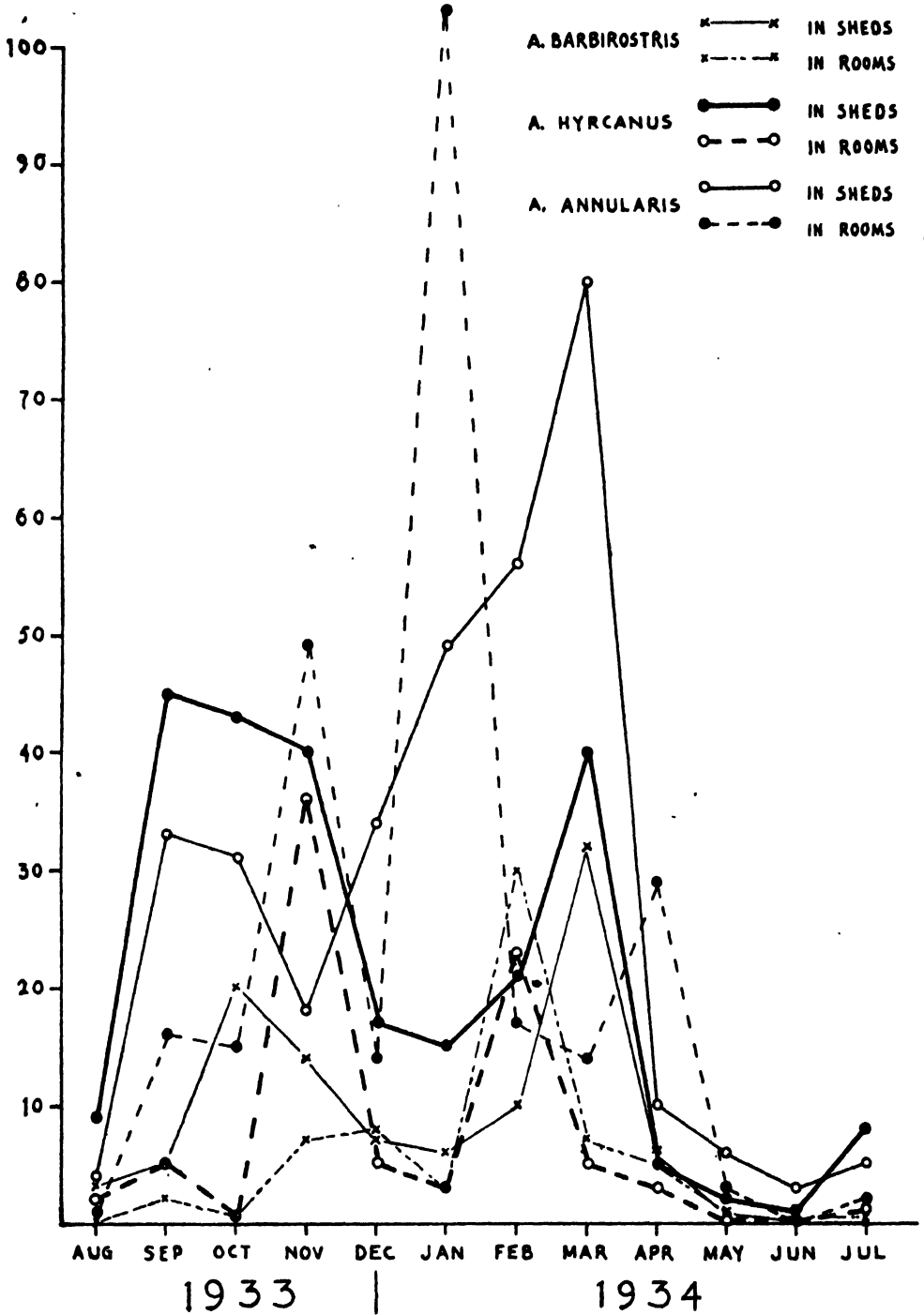
N.B.—Figures in the top row represent total number caught of each species.

TABLE II.
Incidence of anophelines in cattle-sheds.
(Per ten standardised catches.)

	1933.						1934.						Entire period.
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	
<i>A. barbirostris</i> ..	3 2.8	5 4.1	20 17.0	14 11.3	7 7.3	6 6.4	10 9.2	32 15.9	6 8.2	0.4	0.2	0.3	104 7.7
<i>A. hyrcanus</i> ..	9 8.5	45 37.2	43 36.8	40 32.2	17 17.9	15 16.1	21 19.3	40 19.9	5 6.8	2 2.9	1 1.0	8 5.9	246 18.3
<i>A. subpictus</i> ..	29 27.4	3 2.5	..	2 1.6	12 12.6	3 3.2	6 5.5	24 11.9	28 38.3	36 52.2	69 69.0	82 60.7	294 21.9
<i>A. vagus</i> ..	34 32.1	8 6.6	12 10.2	18 14.5	14 14.7	11 11.8	12 11.0	20 9.9	22 30.1	16 23.2	22 22.0	31 22.9	220 16.4
<i>A. sundaticus</i> ..	21 19.8	19 15.7	1 0.8	11 8.9	1 1.0	1 1.0	1 0.9	1 0.5	..	8 11.6	4 4.0	6 4.4	74 5.5
<i>A. annularis</i> ..	4 3.8	33 27.3	31 26.5	18 14.5	34 35.8	49 52.7	56 51.4	80 39.8	10 13.6	6 8.7	3 3.0	5 3.7	329 24.5
<i>A. philippinensis</i>	2 1.6	1 1.0	4 0.39
<i>A. ramsayi</i> ..	4 3.8	5 4.1	3 2.6	6 4.8	2 2.1	2 2.1	1 1.3	2 1.5	26 1.9
<i>A. varuna</i> ..	1 0.9	2 1.7	5 4.2	6 4.8	2 2.1	3 3.2	2 1.8	2 0.9	1 1.3	25 1.86
<i>A. aconitus</i> ..	1 0.9	..	2 1.7	7 5.6	5 5.3	3 3.2	1 0.9	1 0.5	21 1.5
<i>A. culicifacies</i>	1 0.09

N.B.—Figures in the top row represent total number caught of each species.

CHART 1.



It may be gathered from these tables that *A. barbirostris* is a shed-loving species and that this species is rarely found in the months of May to July (*vide* Chart 1). This may be dependent on their breeding being considerably reduced during this period.

A. hyrcanus has also a preference for cow-sheds and its prevalence is much reduced during the months of May and June as shown in Chart 1.

A. subpictus is most abundant from April to July (*vide* Chart 2). This species appears to show a preference for animal quarters, although a considerable proportion may be recovered from dwelling rooms.

A. vagus is fairly prevalent at all times of the year, and does not appear to be much affected by seasonal variations in climate. This species constituted a large proportion of the total catch in dwelling rooms and was the most prevalent species in this locality.

A. sundaicus is most prevalent from May to November and is appreciably reduced in the dry weather (*vide* Chart 2). The percentage of this species caught in houses is not inconsiderable, and it appears to have a distinct preference for this type of resting place.

A. annularis is most prevalent from September to March. Like *A. barbirostris* the adults are scarce during the months of May, June, July and August. Although in some months the proportion of *A. annularis* caught in rooms exceeded that of the cow-shed catches, this species seems on the whole to have a distinct preference for cattle-sheds where, along with *A. vagus*, it accounted for a large proportion of the total adult catch for the year.

A. philippinensis appears to be essentially an autumnal species being frequently represented in the catches made from September to December. This species appears to prefer human habitations since the majority of adults were collected from human dwellings.

A. ramsayi is most prevalent from July to December, and *A. varuna* from August to January, the latter species showing a short rise again in March or April. Both these species are almost equally represented in the adult catches from cattle-sheds and human habitations in this part of Bengal.

A. aconitus appears to be a cold weather species since it was most evident from October to March. In this part of Bengal this species exhibits a somewhat greater inclination to rest in human habitations.

A. culicifacies represented only a very small proportion of the total collection and the few specimens encountered were caught in the months of March, April, and July, mostly from cow-sheds. This species should not be viewed with concern in the causation of malaria in lower Bengal especially in the environs of Calcutta.

When the total catches for the whole year are analysed we find that in dwelling houses the most commonly observed species (in order of prevalence) were *A. vagus*, *A. annularis*, *A. subpictus*, *A. sundaicus*, and *A. hyrcanus*, whereas in the cattle-sheds examined during the same period the predominant species (in order of prevalence) were *A. annularis*, *A. subpictus*, *A. hyrcanus*, *A. vagus*, and *A. sundaicus*.

The ratio of the sexes among the wild-caught anophelines in this locality is shown in Table III. It has been shown in a previous paper (Sen, 1935) that, in laboratory-bred mosquitoes, males usually account for from 40 to 50

CHART 2.

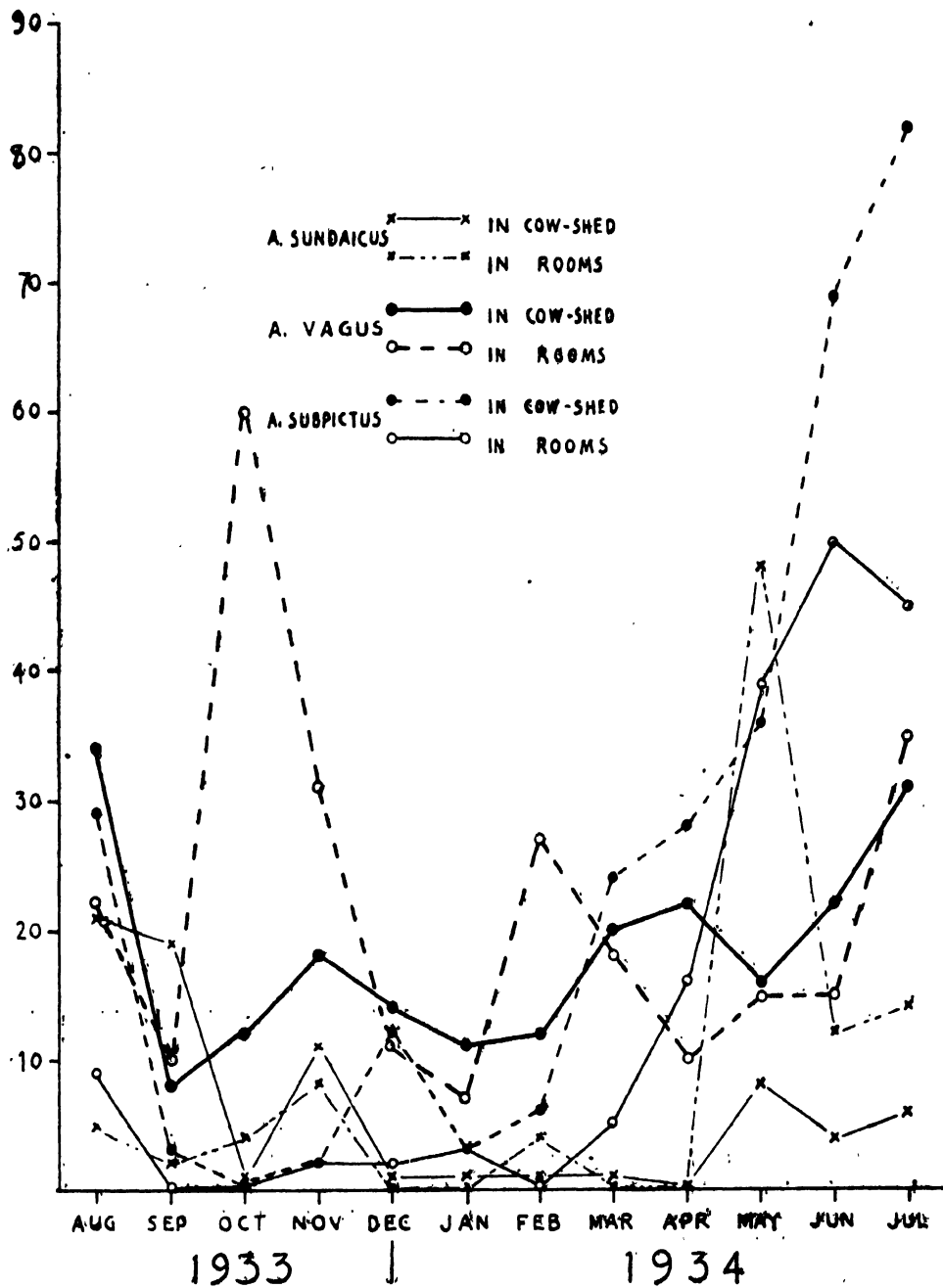


TABLE III.

Ratio of sexes in anophelines caught in cattle-sheds and human habitations.

Species.	Period.	MALES.		FEMALES.	
		Total.	Percentage.	Total.	Percentage.
<i>A. barbirostris</i> ..	August 1933 to July 1934.	29	3.2	881	96.8
<i>A. hyrcanus</i> ..	Do.	114	4.9	2,210	95.1
<i>A. subpictus</i> ..	Do.	824	19.9	3,314	80.1
<i>A. vagus</i> ..	Do.	605		2,439	
<i>A. sundaicus</i> ..	Do.	105	10.7	876	89.3
<i>A. annularis</i> ..	Do.	104	3.2	3,122	96.8
<i>A. philippinensis</i>	Do.	4	5.8	64	94.2
<i>A. ramsayi</i> ..	Do.	12	4.3	264	95.7
<i>A. varuna</i> ..	Do.	25	9.6	234	90.4
<i>A. aconitius</i> ..	Do.	17	7.8	200	92.2
<i>A. culicifacies</i> ..	Do.	1	11.1	9	88.9

per cent of the bred specimens. In the present investigation males were seldom represented in the adult catches and the proportion of males of most species was usually below 10 per cent. The percentage of males in the adult catches was highest in the cases of *A. subpictus*, *A. vagus*, and *A. sundaicus*.

SUMMARY.

1. The relative incidence of anophelines in cattle-sheds and human dwellings in a group of Bengal villages along the banks of the River Hooghly has been discussed in this paper.

2. Anophelines were always much more abundant in the cattle-sheds than in human dwellings close by. No species was absolutely restricted to either type of resting places.

3. Among wild-caught adult anophelines, females were greatly in excess of males. The proportion of males was usually less than 10 per cent of the total catch in a year, but the males of *A. subpictus*, *A. vagus*, and *A. sundaicus* were sometimes present in somewhat greater numbers.

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THE MAXILLARY INDEX OF THE INDIAN ANOPHELINES.

BY

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[18th February, 1937.]

1. HISTORICAL.

THAT differences in the number of teeth on the maxilla of an anopheline might be related to its blood preferences, and that a cyclical change in their number caused by easier access to bovine blood might account for the gradual disappearance of malaria from most of Europe was first suggested by Wesenburg-Lund (1920-21). The first actual investigations were made by Roubaud (1921). He counted these teeth in *A. maculipennis* from various regions of Europe and reached the conclusion that an average count, 'maxillary index', of 14 or less was associated with human blood as food, a count of 14 to 15 with cattle as food, whilst insects with a count exceeding 15, though primarily cattle feeders, could be forced to human blood again through lack of cattle, and that the higher the index the more dangerous was the fauna. A paper by Grassi (1921) provided a brief addendum on this new subject. He found no significant differences in insects from Schito (no malaria) and from Porto (malaria present) nor, among the latter, between insects collected in houses and those collected in pigsties.

This hypothesis was adversely criticised by Martini (1922; 1924). He stated that the number of teeth seems to depend on warmth during larval development, for in any locality individuals bred in warmer water have fewer teeth than have those from colder water. The latter being, on an average, larger will therefore, presumably, have more teeth. De Buck (1926) and Swellengrebel, de Buck and Schoute (1928) disagreed with Martini, but E. de Buen (1935) showed that there is no correlation between the size of a specimen and the number of its teeth.

Roubaud (1928) published the results of eight years' work subsequent to his first papers, and confirmed his critical figures of 14 and 15.

The conclusive way, of course, to test the validity of this hypothesis is by simultaneously counting an insect's teeth and determining the source of its last

blood meal by the precipitin test. This was first done by Yatzenko, Rosenberg and Tishchenko (1930). Their results were distinctly favourable to Roubaud's hypothesis, for they found :—

- (i) The majority of cattle-fed specimens had an index of 14 to 15.
 - (ii) The majority of specimens with both human and bovine blood had an index of 15.
 - (iii) The majority of human-fed specimens had an index of 16 or higher.
- Toumanoff (1935) has definitely confirmed this for several Oriental species, and has further shown that, in an individual species, specimens that average low and high counts show differing percentages that have taken human blood.

Meanwhile doubt had been thrown on Wesenburg-Lund's original hypothesis, which had been accepted by Roubaud, regarding increasing index through increased cattle feeding, leading to the disappearance of malaria, by Ekblom and Ströman (1931). They found the M. I. (maxillary index) of Swedish *maculipennis* to be 17.8 (probably the highest in Europe). From old collections they gathered eight specimens of this species, which had been captured at the beginning of the nineteenth century, and found the index of these to be 17.1. There had thus been no significant change in a century during which malaria had entirely disappeared from Sweden.

Up to this period *A. maculipennis* had been considered as a single species, and the difference in M. I., among other characters, was being invoked to try and account for its very different habits in the various regions of Europe. The evidence adduced was so contradictory that the majority of malaria workers were not inclined to place much faith in the value of the index. But it was at this juncture that the pioneer researches of van Thiel and of Falleroni bore fruit at the hands of Hackett and Missiroli, and the puzzle presented by all work with *maculipennis* fell into place with the distinguishing of the six constituent subspecies. De Muro (1933) was the first to work on the M. I. of the separate subspecies, and the vector *labranchiae* was shown to have an M. I. of 12.8, whilst the non-vectorial *typicus* and *messeae* in the same district had an average index of over 14, thus bearing out the more definite assertion in Roubaud's hypothesis. That the less definite assertion dealing with the androphily of species with counts of over 15 is also probably well founded was shown by the work of Hackett and Missiroli (1935) in Germany, Holland and Italy, though apparently different values and results were reached in the same year by Sergeant and Trenszt (1935), and by Ottolenghi and Rosa (1935), all the subspecies except *melanoon* being covered. But none of this work seems to have been accompanied by precipitin tests.

For twelve years, work on this subject was practically confined to *maculipennis* in Europe. I can only trace two papers on the anophelines of other faunal regions. Shannon (1924) examined a few species of the Nearctic fauna (including the Western American *maculipennis*), and Prado (1929) two Neotropical species, but the first serious work outside Europe, and the first on the Oriental fauna, was that of Morin (1933), followed in the same year by that of Roubaud, Toumanoff and Gaschen (1933), relating to seven species from Indo-China, all of which are also found in British India. The findings in the second of these papers were compared with infection rates in the same species locally, and it at once became apparent that there was very definitely something in the hypothesis of general application, even if Roubaud's absolute

European values were not to be accepted. These papers were followed by others from this group of French workers, as listed in the bibliography that forms the final part of the present paper, and by work on several species (again all found in British India), as found in the fauna of Netherlands-India, by van Thiel (1935).

Recently Wanson (1935) has done the first work on the Ethiopian fauna, showing that though *A. gambiae* has two wing-length types, both have an identical maxillary index of 12.2.

The present writer took up the study for British India in 1933. That it has taken nearly four years to carry through has in large part been due to difficulty in obtaining some of the less common species in adequate numbers.

2. EXPERIMENTAL.

In taking up this work the first point to be settled was the magnification under which the teeth were to be counted. Roubaud (1921), in his original paper, stated that he used a No. 7 objective, make not stated, nor eyepiece magnification, but Toumanoff (1935) states that he follows Roubaud's technique, which is to examine with a No. 7 objective and a comp. ocular 6, giving a magnification of 600-650. Shannon (1924) used only $\times 440$. De Buck (1926) and Swellengrebel, de Buck and Schoute (1928) used an immersion lens, as did also Trenz (1931), who gives details of making preparations. Morin (1933) only stated that his work was done with a 'dry objective'. Van Thiel (1935) used an immersion lens, and states that he thus obtained higher values than by Roubaud's method, and worked out a 'reduced index' for comparison. Few other authors mention the magnification used.

Throughout my work I have used a Zeiss 1/12th immersion, N. A. 1.30, of 90 primary with K7 \times eyepiece, giving a magnification of 630, the specimens being mounted in balsam.

Leaving out Shannon's rather low power work, it would appear that my own, as regards the magnification used, is comparable with that of other workers up to 1934, except Morin. But there are other points to be considered before results by various workers can be compared absolutely. Toumanoff (1935) gives figures illustrating his method, and what he does and does not consider to be a tooth. De Buck (1926) and Swellengrebel, de Buck and Schoute (1928) definitely state that they count a double tooth, i.e., one with two cusps, as a single tooth. My own method is also to count such a tooth as one, but if it is clearly an accessory tooth, as indicated by its irregularly spaced position in the row, but is distinctly separated from those immediately adjacent, then I add it to the count, which is not done by Toumanoff. None-the-less, the number of specimens showing such peculiarities is so few that in an index based on the number of specimens used by me, the error introduced is negligible. A much more probable source of difference, again depending on the opinion of the individual worker, is in the matter of the last tooth of the row, towards the base of the maxilla. By this tooth I do not mean the protuberances lettered 'P' in Toumanoff's text-figure on p. 47 of his paper (appearances which I have never encountered) but in the usually very lightly chitinated last true tooth towards the base. This is often not situated on the heavily chitinated blade of the maxilla, but in the softer, striated, membranous part of the organ. All stages

in the development of a true tooth can be seen here, from a very lightly pigmented aggregation of yellow chitin without a distinct central point, up to the development of a definite denticle. When such can be clearly focused as a sharp point I count it as a tooth, but it is perfectly possible that another worker might not do so. Thus my indices may be + 1 more than those of another worker with the same specimens. Roubaud and Treillard (1934) are now counting at under $\times 700$, which permits them 'to neglect the abortive teeth at the extremities'. This appears to result in an index about 1.6 lower than by the methods of van Thiel and myself, causing the former to work out his 'reduced index'. Roubaud thus does not hesitate to decide which teeth are, and are not, functional, but gives neither arguments nor data for such an assumption, which appears to me to be a dangerous one. It seems far safer to count every denticle which may have a cutting function.

Finally, the numbers on which the index is based must be considered. This vitally important point was examined theoretically by Trenszt (1931). The table on page 207 of his paper is reproduced below, giving the probable error for different numbers counted.

n.	s.e.
25	± 1.0
50	± 0.7
100	± 0.5
200	± 0.3
500	± 0.2
1,000	± 0.15
10,000	± 0.05

Roubaud's original work, and much of that of others, was done with very small numbers, seven to twenty-five specimens, i.e., not more than fifty counts. As no difference is significant unless it is thrice its probable error it is really quite useless to give an index to even one point of decimals, far more so to two, as is done by some authors. Trenszt's table shows how little extra accuracy is gained by increasing the number counted from 200 to 1,000, and it is manifestly impossible to count 10,000 to make the first decimal significant, even were it not likely, as pointed out by Roubaud (1930)* in his reply to Trenszt's paper, that fatigue would introduce more inaccuracy than the use of smaller numbers. Throughout the present work I have, wherever possible, counted 200 maxillæ for a result. This number has a s.e. of ± 0.3 , making a difference of 1 in an index statistically significant. I have also followed Trenszt in counting individual maxillæ, without regard to pairs, thus obtaining results which can be put into smooth curves, though Table III shows that I have throughout obtained at least ninety-six per cent of the maxillæ for any species as actual complete pairs.

Whilst most species can be caught in sufficient numbers as adults without any difficulty, there are certain species which cannot, though they can be bred from larvæ in quantity, e.g., *stephensi* and *maculatus*. It was therefore necessary to find out whether an index prepared from bred specimens was identical with one worked out from wild-caught adults.

* It would appear that Roubaud saw and replied to Trenszt's paper before the latter was published.

Using *A. subpictus*, the following results were found for 200 bred and 200 caught adults from Calcutta, in the month of March :—

		Number of teeth.									
		11	12	13	14	15	16	17	18	19	M. I.
Bred	..	1	4	14	20	58	42	43	16	2	15.6
Caught	1	15	26	47	78	23	8	2	15.5

Though the index is the same, in the caught series there are $12\frac{1}{2}$ per cent more specimens adjacent to the mean than in the bred series. Also the curve for the latter does not fall evenly from the fastigium. Applying the χ^2 test, we get a value of 23.6 with 6 degrees of freedom, indicating that bred and caught insects are significantly different in dispersion, the odds against chance being over 100 : 1.

Two explanations for this fact are possible: (i) that artificial breeding conditions affect the dispersion, and (ii) that in nature there is a considerable production of adults with a dentition far removed from the mean for a species, which fail to feed effectively, and die without appearing as day-resting, engorged specimens. To test these possibilities a similar experiment was made in May, using pupæ collected from natural breeding places and due to emerge within twenty-four hours, to give artificial conditions the minimum chance of affecting the specimens. *A. subpictus* was the species again used, 200 females in each case being counted.

		Number of teeth.										
		11	12	13	14	15	16	17	18	19	20	M. I.
Bred	..	1	6	13	31	53	47	31	13	4	1	15.5
Caught	1	9	19	56	67	36	8	3	1	15.7

Here with 7 degrees of freedom and χ^2 of 13.2 the odds are between 10 : 1 and 20 : 1 against chance, a not very conclusive result. The experiment was therefore repeated in November with the same species and numbers.

		Number of teeth.										
		11	12	13	14	15	16	17	18	19	20	M. I.
Bred	1	6	26	69	57	31	8	2	..	15.6
Caught	12	33	49	54	37	13	1	1	15.6

This gives a χ^2 of 7.6 on 6 degrees of freedom, the odds against chance being no more than 6 : 1.

It was therefore concluded that adults bred from pupæ only differ from adults caught in nature within chance limits, and that therefore the maxillary index of adults bred from pupæ can be compared with that of caught adults. There is no evidence that non-viable adults, with unsuitable dentition, are produced naturally.

This finding, however, is not in accordance with evidence from other workers. Roubaud (1921) records (on ten specimens) a gorged index of 16.6, as against an ungorged of 14.1. De Muro (1933), working with *A. elutus*, found that twenty-eight specimens that oviposited had an M. I. of 13.3*, whilst two hundred specimens that did not oviposit had an M. I. of 14.8. Torres-Canameres (1934), working with *A. maculipennis* (probably *A. atroparvus*), found the index of gorged females to be 15.1, for ungorged 14.7. Thus it is not certain that non-viable adults are not naturally produced.

The next preliminary point to be settled was whether, in making the maxillary index for a species, adults collected at different seasons could be lumped together. If there should be a definite cyclical change in the index, then this cannot properly be done. For this investigation *A. subpictus* was again used, being the only species in Calcutta that can be found in sufficient numbers throughout the year. Two hundred females each month were examined from some buffalo-sheds in Matiabruz (Southern Calcutta) which probably originated from the same breeding places all the year round. The results are given in Table I.

These show that, on raw figures, there is evidence of a double cyclical variation in the index, with minima in April and December, and maxima in August and February. The figures are carried over thirteen months to show the cycle more distinctly.

The only meteorological factor that appears to have any relationship to this variation in index is the rainfall. There appears to be no relationship with maximum, average or minimum temperatures, or with saturation deficiency. Heavy rain, and equally, long-continued lack of it, both seem to have the effect of raising the index. The latter effect is suggested by Galliard and Sautet (1935) working with *labranchiae* and *elutus* in Corsica. They found for these species an M. I. of 12 to 14 in the summer epidemic season, and an M. I. of 13 to 16 in winter, and suggest that this correlates with ample breeding facilities at the beginning of summer, whereas in September, before the first rains, breeding is reduced to a minimum and only robust individuals survive, leading to hibernators with a high M. I.† As regards 'first rains', September in Corsica corresponds with March in Calcutta, which follows three or four almost rainless months.

But this explanation is directly contrary to the facts as regards the rise of the index in Calcutta during the rainy season. The rise is a real one, statistically significant, amounting to a whole tooth as between April and August, which is more than thrice the probable error of the observations, with a χ^2 of

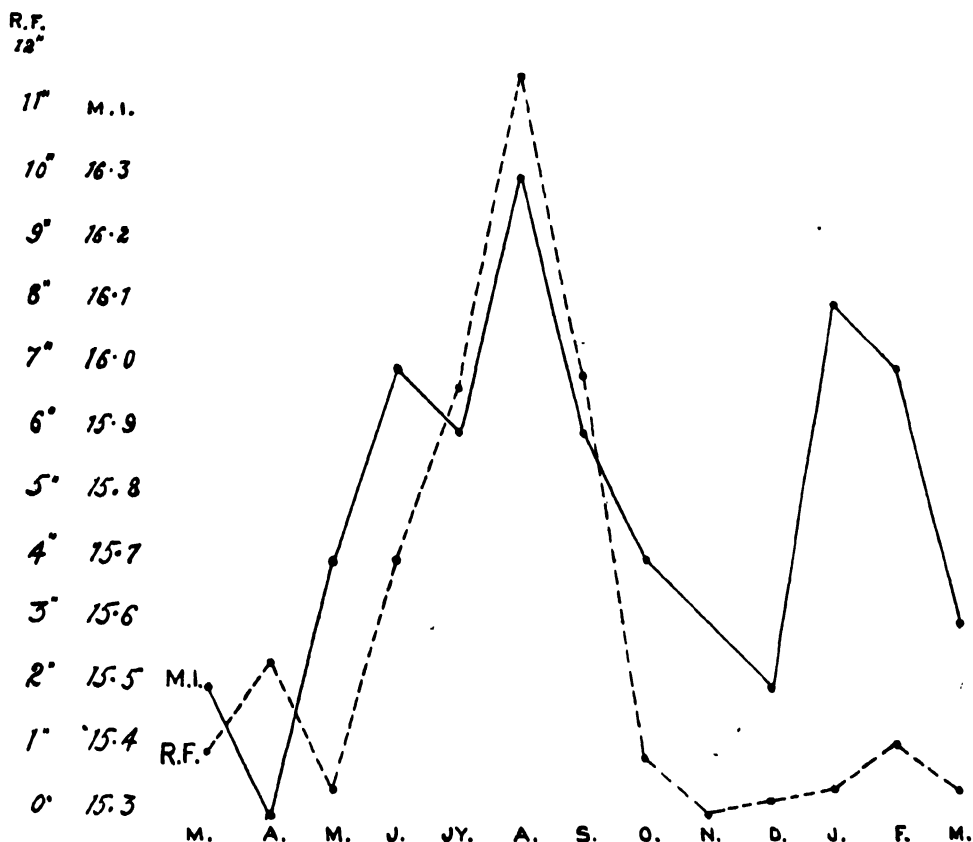
* There is an error in the text, but not in the tables, regarding this figure.

† As pointed out previously, E. de Buen has shown that there is no correlation between size and M. I.

TABLE I.
A. subpictus—monthly maxillary counts in Calcutta.

Month.	Average maximum temperature.	Average minimum temperature.	Average saturation deficiency 8-00 hrs.	Total rainfall in inches.	Days.	Number of teeth.										Total.	Maxillary index.
						12	13	14	15	16	17	18	19	20			
1935.																	
March ..	93	72	4.0	1.0	2	1	15	26	47	78	23	8	2	..	200	15.5	
April ..	95	75	5.3	2.4	6	6	15	29	55	57	29	6	3	..	200	15.3	
May ..	100	85	7.6	0.4	3	1	9	19	56	67	36	8	3	1	200	15.7	
June ..	95	81	6.3	4.0	10	..	2	19	51	57	49	18	2	2	200	16.0	
July ..	90	80	3.6	6.7	18	20	55	69	36	17	3	..	200	15.9	
August ..	89	79	3.0	11.6	17	..	3	13	45	51	54	26	7	1	200	16.3	
September	89	79	3.3	6.9	16	..	6	16	52	63	48	11	3	1	200	15.9	
October ..	91	74	4.5	0.9	2	1	11	26	52	51	35	20	3	1	200	15.7	
November	86	63	3.7	0.0	0	..	12	33	49	54	37	13	1	1	200	15.6	
December	80	57	2.3	0.2	1	1	11	28	67	52	33	6	2	..	200	15.5	
1936.																	
January ..	79	56	3.2	0.4	1	..	3	15	56	51	48	22	3	2	200	16.1	
February ..	84	61	1.7	1.1	1	1	6	15	47	61	46	19	4	1	200	16.0	
March ..	96	72	5.7	0.4	1	2	9	25	53	63	38	5	5	..	200	15.6	

GRAPH.

Calcutta maxillary index of *A. subpictus* and rainfall.

22.9 on 6 degrees of freedom, making odds of over 100 : 1 against chance. It is impossible to invoke in explanation Roubaud's (1921) hypothesis that a less well nourished fauna has fewer teeth, for with the species reaching its numerical maximum (as regards prevalence) in the rains it stands to reason that it must be under optimal breeding conditions. It is, however, in accordance with the suggestion of Torres-Canamares (1934), who explains a considerable decrease found in separate years by Roubaud and by himself at Camporredondo by postulating that the index falls with decreasing competition for food, owing to reduction of breeding in a dry year. On this the index would rise with increasing competition for adult food (assuming the latter to be constant in quantity), when breeding output was maximal, which is directly opposite to the explanation of Galliard and Sautet referred to above.

The problem of cyclical fluctuation in the index, and its cause, is thus seen to be entirely unsolved. None-the-less, the phenomenon has been shown to exist, and it directly affects the question posed as the second preliminary point.

However, I fear the question must be begged, for many of the species to be dealt with in this paper are very seasonal in their appearance at all, even more so in their appearance in numbers, and with some it has taken years to collect two hundred maxillæ. Most of my *A. jamesi* specimens, for instance, were obtained only in October and November 1935, during an unusual prevalence of this generally sparse species in the Satpura Range. Though, therefore, a maxillary index based on collections made where and as available is shown not to be entirely trustworthy, it is, for the rarer species at least, the only course possible.

The last preliminary question to be posed is—is it safe to work out the maxillary index of any species over a wide area of distribution? Though this paper is on 'The Maxillary Index of the Indian Anophelines', the work, unless otherwise specifically mentioned*, has been done in a triangular area of India with apices at Jubbulpore (Central Provinces), Vizagapatam, (Madras) and Calcutta (Bengal). This is a relatively small portion of the Indian sub-continent, though it stretches over five degrees of latitude and eight degrees of longitude. To test whether it would be safe to work out an average M. I. for a species over such a stretch of country, parallel counts were made with *A. subpictus* caught in April and May from breeding in a brackish back-water at Vizagapatam, and caught from breeding in July and August on the black-cotton soil of the Central Provinces at Dongargarh, in Khairagarh State, 270 miles from the nearest point on the coast and nearly 200 miles due north of Vizagapatam, as representing the extremes of breeding conditions encountered in this area of India. The findings were as under :

		Number of teeth.										M. I.
		11	12	13	14	15	16	17	18	19	20	
Vizagapatam	1	7	22	55	55	36	20	4	..	15.8
Dongargarh	..	1	1	9	33	52	59	34	8	2	1	15.6

χ^2 with 7 degrees of freedom is less than 4, showing that any differences are entirely due to chance. As regards the lumping for calculation of specimens from the remotest portions of the area under consideration, this can therefore safely be done.

There is however considerable evidence that there are geographical limits to an M. I. worked out for any species. Shannon (1924) found an average increase of two teeth between Western American *maculipennis* from California and from British Columbia. Roubaud, Toumanoff and Gaschen (1933) point

*The following exceptions to this have occurred:—

A. tessellatus, to complete 200 counts, 4 specimens from Mudigere, Mysore State, were examined. Their counts fell well within the normal for the species in 'East Central India'.

A. karwari, only 10 specimens have been obtained from mv area in the last four years. The balance counted were all from Mudigere, Mysore State. These Mysore specimens were obtained through the kindness of Dr. W. C. Sweet, of the Rockefeller Foundation.

There are three species occurring in mv area of which no examinations have been made, viz., *A. aikenii*, *A. majidi*, and *A. moghulensis*. They are all so rare that there is no hope of obtaining sufficient specimens for their M. I. to be worked out.

out that for *A. sinensis*, *A. vagus*, *A. minimus*, and *A. aconitus* in Indo-China the index rises slightly from Tonkin to North Annam and Laos, though as the numbers counted are not given it is doubtful if the increase found is significant, for though Ma and Chang (1935) counted 1,000 maxillæ for the first of these species from around Shanghai, yet the further rise over the north Indo-China figure that they show is not statistically significant. Certainly significant, however, are the large differences found by Hackett and Missiroli (1935) for two European races of *maculipennis* in various countries :—

		<i>messeae</i>	<i>atroparvus</i>
Germany	17	17·5 to 17·8
Holland	16 to 17	17 to 18
North Italy	15·7	15·8
Central Italy	15·4	..

Compare for these species the findings for France, situate as regards latitude between Holland and North Italy, by Sergent and Trenszt (1935), who found an average of 15·6 for *messeae* in Alsace, and of 16 to 17 for *atroparvus* in the Limousin. The latter figure is in strict conformity with a gradual rise in M. I. towards the north. None-the-less, from Peshawar in the North-West Frontier Province to Hambantota in Ceylon is a matter of twenty-eight degrees of latitude, and it would be rash to assume that my findings given below for what is best called 'East Central India' will be found uniform throughout the sub-continent.

3. OBSERVATIONS.

The results of the present investigation are given in Table II.

The species are arranged in systematic order. The index is given both as computed by dividing the total number of teeth counted by the number of maxillæ examined, in which, as has already been shown, the decimal point is insignificant, and as the nearest whole number, which it is more correct to use in any discussion. Grouping in this fashion, however, reveals no sort of systematic relationships other than between a few pairs or triplets of species.

In the table below the species are again tabulated under their whole-number indices, with those that have been found naturally infected within the area investigated shown in bold face type. For this purpose the finding of oöcysts has been taken as the criterion indicating the source of the blood meal, and not of sporozoites, the finding of which may be a matter related to the life of the species after the first blood meal.

MAXILLARY INDEX.

12	13	14	15	16
<i>A. fluviatilis</i>	<i>A. tessellatus</i>	<i>A. nigerrimus</i>	<i>A. vagus</i>	<i>A. subpictus</i>
<i>A. minimus</i>	<i>A. oullofacies</i>	<i>A. barbirostris</i>		
<i>A. varuna</i>	<i>A. jeyporensis</i>	<i>A. sundalcus</i>		
<i>A. aconitus</i>	<i>A. stephensi</i>			
<i>A. ramsayi</i>	<i>A. maculatus</i>			
<i>A. splendidus</i>	<i>A. theobaldi</i>			
<i>A. pallidus</i>	<i>A. karpuri</i>			
	<i>A. jayesi</i>			
	<i>A. annularis</i>			
	<i>A. philippinensis</i>			

TABLE II.
Maxillary counts.

Species.	Number of teeth.										Num-ber of maxil-lae counted.	Maxil-lary index com-puted.	Index value.			
	8	9	10	11	12	13	14	15	16	17				18	19	20
<i>A. nigerimus</i>	2	8	38	68	58	16	9	1	200	14-3	14
<i>A. barbirostris</i>	8	15	52	74	37	12	2	200	13-8	14
<i>A. tessellatus</i>	..	1	1	17	56	80	37	8	200	12-8	13
<i>A. culicifacies</i>	1	16	60	67	43	13	200	12-9	13
<i>A. fluviatilis</i>	1	2	15	37	51	56	28	9	1	200	12-3	12
<i>A. minimus</i>	..	1	11	57	55	64	10	2	200	12-0	12
<i>A. varuna</i>	9	37	86	52	15	1	200	12-1	12
<i>A. aconitus</i>	..	1	5	36	74	62	16	4	2	200	12-3	12
<i>A. jeyporiensis</i>	3	25	51	72	45	4	200	12-7	13
<i>A. subpictus</i>	1	1	15	26	47	78	23	8	2	..	200	15-5	16
<i>A. vagus</i>	4	4	11	41	56	50	28	6	3	..	200	15-3	15
<i>A. sondaicus</i>	4	29	47	57	43	16	4	1	200	13-9	14
<i>A. stephensi</i>	6	23	51	59	36	12	7	4	1	..	1	200	12-9	13
<i>A. maculatus</i>	3	27	57	56	36	17	4	4	200	12-8	13
<i>A. theobaldi</i>	5	27	65	67	26	8	2	200	12-6	13
<i>A. karwari</i>	1	6	13	34	26	14	1	1	96	13-3	13
<i>A. jamesi</i>	5	35	63	60	31	5	1	200	12-5	13
<i>A. ramsayi</i>	..	1	16	79	79	23	2	200	11-5	12
<i>A. splendens</i>	..	4	24	62	69	28	10	2	1	200	11-7	12
<i>A. annularis</i>	7	32	63	63	28	7	200	12-5	13
<i>A. philippinensis</i>	6	34	44	64	39	11	2	200	12-7	13
<i>A. pallidus</i>	12	25	77	56	25	5	200	12-4	12

In this grouping, though there are anomalies, the relationship between maxillary index and natural infection is very striking. In the M. I. 12 group *A. splendidus* is not in bold face type, since it has not been found infected in the area investigated. Robertson (Covell, 1927) however found it infected in Saharanpur; on the other hand Ramsay, Chandra and Lamprell (1936) have shown that in their area, at least, it seems never to bite man. However, it is highly significant that all the seven species in this group have been found naturally infected somewhere in India.

In the M. I. 13 group four species out of ten have been found with oöcysts in the area investigated. Two others, *A. stephensi* and *A. maculatus*, are carriers in other parts of India. *A. karwari* has once been found with a gut infection in Cachar (Covell, 1931).

But as regards *A. stephensi* there are several points to be discussed. In the first place, it is well known that, in Calcutta, *A. stephensi* is almost impossible to find as a resting adult. The specimens used in my work were from the stock maintained at the Calcutta School of Tropical Medicine in connection with the Malaria Transmission Enquiry of Knowles and Basu (not yet published owing to the lamented death of Colonel Knowles in 1936). This stock was under sufficiently natural conditions in a big Martini-pattern breeding cage to survive for three generations without renewal, but it was not under entirely natural conditions, as it could not be carried beyond the third generation. It carried malaria well under laboratory conditions, but so will many other species that have never been found infected in nature. The M. I. in Table II is worked out, therefore, from bred-out larvæ, and though probably close to the natural figure is most probably not comparable as regards dispersion with what occurs in nature, as the experiment with *A. subpictus* on page 159 shows.

As Ramsay and Macdonald (1936) have also shown, the habits of *A. stephensi* as an adult in Western and in Eastern India are quite different, and these authors quote the suggestion of Knowles and Basu (1934) that the species in Calcutta is possibly naturally zoophilic. No species in this country more urgently requires further investigation than *A. stephensi*.

For comparison, therefore, I obtained through the kindness of Dr. W. C. Sweet a number of heads of *A. stephensi* caught in nature, from Mysore. There, at Hiriur, the species breeds in a river, in this respect differing entirely from its urban relatives. It does, however, at times feed upon man, for in 1,776 dissections (Covell, 1931; Nursing, Rao and Sweet, 1934), it has thrice been found with oöcysts. The findings for these specimens were:—

Number of teeth.

11	12	13	14	15	16	17	Total.	M. I.
9	26	49	33	21	5	1	144	13.3

By the χ^2 test these figures appear significantly different from those for *A. stephensi* in Calcutta, and thus do not afford any additional justification for underlining the Calcutta race as a human feeder in the M. I. 13 group.

In the M. I. 14 group we have one most efficient carrier, and two non-carriers, at least as regards this country. The anomalous position of *A. sundaicus*, away from the other vectors, has been commented on by van Thiel (1935) for the Dutch East Indies, where it exhibits exactly the same discrepancy. It is the highest of the paucidentate group, and being a notorious carrier, affords no explanation of why so many of the M. I. 13 group are not found to be so. The only suggestion offered in explanation of this fact is that of Toumanoff (1935) that paucidentateness is associated not only with human feeding, but also with buffalo feeding. Toumanoff's precipitin tests were made with an anti-buffalo serum, not anti-bovine. I understand, however, from Major S. S. Greval, I.M.S., Imperial Serologist, that both cattle and buffalo blood react with the same anti-serum, but in differing lengths of time. Probably one needs to be an expert serologist to distinguish the two reactions. Toumanoff's hypothesis, therefore, appears to be purely speculative and unsupported by valid laboratory evidence.

None-the-less, it is seen that the Indian carrier anophelines, without exception, conform to Roubaud's paucidentate ($\angle 14$) group, but in this group there are so many non-carriers that one cannot seriously entertain the suggestion of Roubaud, Toumanoff and Gaschen (1933) that, in taking up the study of an unknown fauna, the working out of the maxillary index is more valuable than the actual discovery of infected specimens!

TABLE III.

Differences in the number of teeth on the maxilla of either side in individual specimens.

Species.	Difference equals						Number of pairs counted.
	0	1	2	3	4	5	
<i>A. nigerrimus</i> ..	25	57	16	1	99
<i>A. barbirostris</i> ..	41	41	11	6	99
<i>A. tessellatus</i> ..	41	43	11	3	1	..	99
<i>A. culicifacies</i> ..	44	46	5	2	..	1	98
<i>A. fluviatilis</i> ..	32	41	20	2	1	..	96
<i>A. minimus</i> ..	40	41	16	1	98
<i>A. varuna</i> ..	43	42	14	99
<i>A. aconitus</i> ..	33	52	9	4	1	..	99
<i>A. jeyporiensis</i> ..	40	48	11	99
<i>A. subpictus</i> ..	30	40	19	7	2	1	99
<i>A. vagus</i> ..	35	42	13	5	4	..	99
<i>A. sundaicus</i> ..	24	45	21	5	1	..	96
<i>A. stephensi</i> ..	45	34	9	9	1	..	98
<i>A. maculatus</i> ..	33	45	17	4	99
<i>A. theobaldi</i> ..	29	42	24	2	97
<i>A. karwari</i> ..	21	18	7	2	48
<i>A. jamesi</i> ..	34	50	11	1	96
<i>A. ramayi</i> ..	39	45	13	97
<i>A. splendendus</i> ..	40	43	12	2	97
<i>A. annularis</i> ..	34	49	11	2	96
<i>A. philippinensis</i> ..	33	40	22	3	98
<i>A. pallidus</i> ..	39	39	17	1	1	..	97

Finally, it remains to compare findings within the Oriental region for species which have been examined in more than one country. In Table IV these are given, taken for British India from Table II, and for French Indo-China and the Dutch East Indies from Toumanoff (1935) and van Thiel (1935). From the latter the 'reduced index' referred to on page 157 is also given. The agreement between van Thiel and myself for all species except *A. aconitus* is very close. So is also the Dutch author's reduced index with those found by Toumanoff, except in the case of *A. tessellatus*. It would thus appear that an average addition of 1.6 should be made to indices found by the French method to make results comparable with those found in other parts of Asia. On the other hand the agreement of the uncorrected French indices with my own is very close in the case of *A. splendidus* and *A. sundaicus*. Such discordant results are very puzzling, unless it can be shown that certain species at least have different indices in different countries, which would certainly seem to be the case with *A. subpictus* in Tonkin and in Indo-China.

TABLE IV.

*Maxillary indices in various countries of the Oriental region.**

Species.	British East Central India.	West Indo- China.	South Indo- China.	Tonkin	Cochin- China.	DUTCH EAST INDIES.	
						Found.	Reduced.
<i>A. nigerrimus</i> ..	14.3	14.7	13.1
<i>A. barbirostris</i> ..	13.8	13.8	12.2
<i>A. tessellatus</i> ..	12.8	12.1	12.4	12.8	11.2
<i>A. minimus</i> ..	12.0	11.5	11.3
<i>A. aconitus</i> ..	12.3	11.3	11.3	13.2	11.2
<i>A. subpictus</i> ..	15.5	13.0	14.3	15.3	13.7
<i>A. vagus</i> ..	15.3	14.1	14.4	15.8	14.1
<i>A. sundaicus</i> ..	13.9	13.7	14.5	12.9
<i>A. maculatus</i> ..	12.8	11.3	13.2	11.6
<i>A. karwari</i> ..	13.3	12.6	11.0
<i>A. splendidus</i> ..	11.7	11.6	11.8
<i>A. annularis</i> ..	12.5	11.4	11.3	12.7	11.1
<i>A. philippinensis</i>	12.7	11.4	11.4

4. MISCELLANEOUS.

There is equality in the number of teeth on the two maxillæ of an anopheline in less than 50 per cent of cases. Table III gives the figures as regards Indian anophelines dealt with in this paper, in as many of the specimens as yielded complete pairs on dissection.

Though there is no present value in these figures, they are recorded since they may be of value to some future worker on the subject. Sergeant, Ed. and Et., Parrot and Foley (1922) note that in Algiers the difference in *A. maculipennis* may amount to three teeth in a pair of maxillæ. Trenszy (1931) states that in 400 specimens of *A. maculipennis* 35 per cent were equal, 44 per cent differed by one tooth, 16 per cent by two teeth, 4 per cent by three and 1 per cent by four. Torres-Canamares (1934) in Spanish *A. maculipennis* observed

differences of up to four teeth. Ma and Chang (1935) with *hyrcanus sinensis* found differences of up to five teeth.

The Oriental anophelines therefore present nothing peculiar, though some species are more constant in this respect than others.

ACKNOWLEDGMENTS.

In conclusion I have to thank Professor P. Buxton for much helpful criticism regarding the statistical side of this enquiry. I must also record my indebtedness to my senior Laboratory Assistant, Babu M. Lazarus, without whose work in making the thousands of mounts counted it would have been quite impossible for me to have taken up this study as a spare time investigation.

ANNOTATED BIBLIOGRAPHY.

Owing to the language difficulty—comparatively few of the papers referred to are in English—the study of the maxillary index has not been taken up by any other worker in this country. The annotated bibliography given below is believed to be complete for the subject up to the year 1935.

1920-21.

1. Wesenburg-Lund. 'Contributions to the Biology of the Danish Culicidæ'. *D. Kgl. Danske Vidensk. Selsk. Skrifter, Nat. and Math., Afd. 8, Raekke, VII*, (1).

Page 194. Discussing disappearance of malaria from Denmark between 1830 and 1900 suggests, following Roubaud, a more thorough examination of the maxillary teeth.

The double date given for this paper is that on the title page.

1921.

2. Grassi. 'Nuovo Orizzonte nella Lotta Antimalarica'. *Riv. di Biologia*, III, (4), 421-463.

M. I. is treated only in an addendum. *Anopheles* from Schito, 108 examined, M. I. 13.76. From Porto (in houses), 107 examined, M. I. 13.44. From Porto (in pigsties), 107 examined, M. I. 13.43.

3. Roubaud. 'La différenciation des races zootrophiques d'*Anophèles* et la régression spontanée du Paludisme'. *Bull. Soc. Path. Exot.*, XIV, 577-595.

Table, page 581, only 7 to 25 specimens examined from each locality. Malaria when M. I. is 13 to 14 and 15.5 to 16.9. States he is following the suggestion of Wesenburg-Lund, so this is the first actual counts' paper. Uses all teeth visible under a No. 7 obj. Finds wide variation ranges, 10 to 15 from Corsica and 12 to 18 from Macedonia. A less well nourished fauna has fewer teeth. Finds (on 10 specimens), M. I. 14.3 not gorged, against 16.6 gorged (in Vendée av. 15.6). Regions with highest range are most dangerous, because of intense concurrence with animals, which are insufficient in numbers. *Anopheles* are only harmless between 14 and 15. Below this are purely human feeders, above this secondarily forced to humans through lack of cattle. The higher the index the more dangerous the fauna.

1922.

4. Langeron. 'Sur l'Anophélisme et le Paludisme en France'. *Bull. Soc. Path. Exot.*, XV, 30-36.

Throws doubt on Roubaud's work owing to the small numbers examined.

5. Martini. Kritische Bemerkungen zur Theorie der 'misanthropen' oder 'zoophilen' Anophelen. *Arch. Schiffs-u. Trop. Hyg.*, XXVI, (9), 257-265.

Criticises No. 3. Individuals developing at low temperature are larger, and will *probably*, on the average, have more teeth. No actual counts in support.

6. Roubaud. 'A propos des races zoophiles d'Anophèles'. *Bull. Soc. Path. Exot.*, XV, 36-39.

Anopheles cannot obey their preferences completely when the influence of extreme hunger drives them to blood from distant breeding places. Concordance only occurs when there are stabilised breeding places close to a choice of hosts. This condition arises from agricultural improvements involving increase of cattle.

7. Sergeant, Ed. and Et., Parrot and Foley. 'L'armature maxillaire des *A. maculipennis* en pays paludéen'. *Bull. Soc. Path. Exot.*, XV, 29-30.

Find the theory inapplicable to Algeria. Difference from one to the other of a pair of maxillæ may be 3 teeth.

In a footnote to No. 11 Roubaud says the enumeration was not done in his way, so is incomparable.

1924.

8. Martini. 'Über jugoslawische Anophelen mit besonderer Berücksichtigung der Frager der misanthropen Rassen'. *Arch. Schiffs-u. Trop. Hyg.*, XXVIII, (6), 254-265.

A. elutus has fewer teeth than *A. maculipennis*. The number of teeth seems to depend on warmth during development, for if both spp. occur in the same locality such individuals as develop in warmer water will have less teeth than those that have developed in colder water. Number of teeth is therefore not a racial character but a climatic modification. Summer generations of both spp. are smaller, on an average, than spring ones, and it is probable that the smaller number of teeth is simply due to smaller size. As malaria is rarer in cooler regions, there is a general, inverse, parallelism between the number of teeth in a given sp. and the decrease of malaria.

9. Shannon. 'Notes on the maxillary teeth of *Anopheles*'. *Proc. Ent. Soc. Wash.*, XXVI, (5), 142-143.

Used $\times 440$ magnification. *A. maculipennis* 19 American specimens from California, M. I. 13 to 19. From British Columbia, 15 to 21. Variations of up to two teeth in an individual. Decrease is usually within the row. In two specimens out of 19 one maxilla missing. The average of the California specimens is 15.3. Thinks counting is not of much value. Chico (Cal.) is a malarious region, but the M. I. is what Roubaud would claim as zoophile in France. Count is higher, 17.3 av., in non-malarious regions of N. America. Queries Roubaud's assumption that man was the original host and 13 the original M. I., and that extra teeth came with increasing zoophilism. Cattle were possibly numerous ages before man came as a rival source of food, therefore cattle are instinctively preferred.

Other spp. examined:—

A. quadrimaculatus from Arkansas, 10 specimens, 13 to 19, av. 15.6.

A. punctipennis from Maryland, 10 specimens, 13 to 17, av. 15.3.

A. eiseni from Canal Zone, No. of specimens not stated, 13 to 14.

A. barberi from Maryland, 1 specimen, 11 to 12.

50 per cent of the *punctipennis* averaged 15 to 16.

A number of Sabethids ranged 6 to 10.

1926.

10. De Buck. 'De variatie bij *A. maculipennis* in verband met het Anophelisme zonder Malaria'. [Thesis. Abstract in *Rev. App. Ent.*, (B), XV, 21.]

Discovery of large paucidentate and small multidentate types in Holland. The latter carries malaria.

1928.

11. Roubaud. 'Nouvelles Recherches sur l'évolution zoophile des faunes d'Anophèles en Europe, (*A. maculipennis*) d'après les données de l'armement maxillaire'. *Ann. Inst. Past.*, XLII, 553-618.

Eight years' work (accounts for the gap in the *Bull. Soc. Path. Exot.* papers), 'Stabilisation of the conditions of life of mosquitoes is one of the most powerful factors for certainty of the action of animal deviation. It is by stability afforded to their biological cycle that mosquitoes come more and more to select their zootrophic preferences, and it is thus, thanks to the factor of hydrologic regulations, that they gradually build up their purely zoophilic races'.

14 is the limiting value between man and animal biters to be used in anti-malaria work. Above 15 man biting begins again. States that the observations of Swellengrebel, van Thiel and Martini are done on the same lines and are comparable. Work on the indices of gorged and ungorged females. Does not count accessory teeth.

12. Swellengrebel, de Buck and Schoute. 'Studies of Anophelism without malaria in the vicinity of Amsterdam'. *CH/Mal.* 72 (undated). Apparently reprinted in *Verh. Kon. Akad. Wetensch. Amst. Afd. Natuurk.*, XXXVII, (4), 386-394, (1928).

Counts made under an oil-immersion. Double teeth counted as single. Differences may be up to 4 in a pair of maxillae. In *A. maculipennis* the M. I. is the same in houses and in stables. Disagree with Nos. 5 and 8 regarding differences in M. I. in this species as a temperature phenomenon. Chlorine content of water does not explain it either. Gives M. I. for other regions of Europe. In Holland, however, M. I. does rise in summer.

1929.

13. Prado. 'Zootropismo dos Anopheles'. *Scientia Medica*, VII, 378-381.

Under 14 are non-zoophile. 14 to 15 are zoophile. Over 15 is badly defined. *A. brasiliensis* has M. I. 14. *A. cruzi*, M. I. 10, from Sierra del Mar, where zoophilic adaptation is impossible, as cattle absent. This is a malaria zone (but there is no statement that this sp. is the carrier).

14. Swellengrebel. 'La dissociation des fonctions sexuelles et nutritives, (dissociation gono-trophique), d'*A. maculipennis* comme cause du paludisme dans les Pays-Bas et ses rapports avec l'infection domiciliaire'. *Ann. Inst. Past.*, XLIII, 1370-1389.

Gonotrophic dissociation, 11,612 examined. M. I. 18.0. Transmitter. Gonotrophic concord, 8,665 examined. M. I. 17.0. Non-transmitter.

1930.

15. Roubaud. 'Quelques remarques à propos de l'interprétation théorique des index maxillaires'. *Bull. Soc. Path. Exot.*, XXIII, 47-53.

A reply to No. 18. Considers Trens's mathematical analysis unnecessary. States that doing 10,000 would be more inaccurate than were smaller numbers examined. Less than 100 mosquitoes will give valuable figures. Stresses necessity for strict uniformity in examination. Finds, under No. 7 obj., as used in original paper, three insects = 15, and under a 1/18th, same insects = 16.1. States he uses a No. 7 in all his work.

16. Yatzenko, Rosenberg and Tishchenko. 'Contribution to the study of the number of maxillary teeth of *A. maculipennis* Mg. in connection with the analysis of the type of blood by means of the precipitin test'. *Sanitent. Byull.*, I, 3, 16-19. [Only seen in abstract trans. in *Rev. Appl. Entom.*, (B), XVIII, 255].

1931.

17. Ekblom and Ströman. 'Geographical and biological studies on the Swedish anophelines from an epidemiological point of view'. *C. R. 2nd. Intl. Mal. Congr., Algiers*, I, 256-276.

A. maculipennis in Sweden. 2.3 per cent only with 14, rest with 15 to 27. M. I. 17.8, higher than in any other country in Europe. Not due to shortage of other hosts than man, but to latitude (*apud* Martini). Eight old specimens collected at beginning of nineteenth century were 16 to 19, M. I. 17.1, so no change in M. I. in last 100 years. Kling showed there was much malaria in Sweden up to 1860, but now there are only imported cases.

18. Trens. 'L'index maxillaire d'*A. maculipennis* et la théorie du zootropisme anophélien'. *C. R. 2nd. Cong. Internat., Algiers*, I, 155-224.

Immersion lens counts. Statistical theory of results with standard error of mean of various numbers counted. Gives differences in 400 specimens from side to side. Single, isolated maxillæ must be considered, not individual insects. Concludes that Roubaud's hypothesis is shown mathematically to be based on too few specimens to be valid.

1932.

19. Roubaud. 'Recherches sur les variations trophiques et biologiques des peuplements de l'*A. maculipennis*'. *Bull. Soc. Path. Exot.*, XXV, 755-762.

Proves paucidentate forms attack man rather than animals. No new data.

20. Roubaud. 'Les races trophiques de l'*A. maculipennis* decellés par les élevages expérimentaux comparés'. *C. R. Acad. Sci.*, CXCIV, 1694-1696.

Paucidentate (13 to 14) hemophages and multidentate (>15) zoophiles breed true over three generations. Cross breeding not tried.

1933.

21. De Muro. 'Sulle diverse razze di *A. maculipennis* nell'Agro Pontino'. *Revista Malariologia*, XII, (1), 98-107.

A. labranchiae, M. I. 12.79, is chief vector in interior of region.

A. messeae, M. I. 14 in 38 out of 60 specimens.

A. typicus, M. I. 14.1.

A. elutus that oviposited (28 layings) had an M. I. of 13.3 (error in text, not in table), whilst 200 that did not lay had an M. I. of 14.8. Coastal *A. elutus* carrying in Pontinia has an M. I. > 14.

22. Morin. 'Au sujet de l'indice maxillaire des Anophèles de l'Indochine Septentrionale'. *Bull. Soc. Path. Exot.*, XXVI, 293-300.

Work done with a dry objective. A convention to count only as teeth those of which the base 'fait corps' with the maxilla. States that in searching for a carrier in a complex fauna M. I. serves to orient work in looking for the major local vector, by first finding the local paucidentate species.

A. vagus 1,225.5 examined. M. I. 14 σ 2.6.

A. sinensis 826.5 examined. M. I. 15 σ 2.29.

A. minimus 483 examined. M. I. 11 σ 2.6.

A. aconitus 93 examined. M. I. 11 σ 2.27.

A. jeyporiensis 153 examined. M. I. 11 σ 1.70.

Points out that the M. I. of *A. aconitus* and *A. minimus* being equal does not necessarily indicate a common origin, as *A. jeyporiensis* has the same index, showing a special correlation for this character.

23. Roubaud and Gaschen. 'Insuffisance des caractères de l'œuf pour la distinction des races trophiques et biologiques de l'*Anopheles maculipennis*'. *Bull. Soc. Path. Exot.*, XXVI, 447-451.

Egg characters are insufficient for distinction of races. Use M. I.

24. Roubaud, Toumanoff and Gaschen. 'Les données de l'indice maxillaire rapportées au rôle infectant des anophèles de l'Indochine septentrionale'. *Bull. Soc. Path. Exot.*, XXVI, 282-293.

Species.	No. max. ex'd.	M. I.	Inf. rate.
<i>A. sinensis</i> 1,090	15.6	0.0
<i>A. vagus</i> 1,595	14.2	0.15
<i>A. minimus</i> 731	11.6	2.17
<i>A. aconitus</i> 266	11.3	1.06
<i>A. jeyporiensis</i> 183	11.8	2.25
<i>A. maculatus</i> 53	11.5	3.20
<i>A. philippinensis</i> 60 (about)	11.4	0.0

Another table gives the M. I. of actually infected specimens.

The M. I. rises slightly from Tonkin to N. Annam and Laos.

The authors suggest that M. I. is more valuable than actual discovery of infection.

25. Weyer. 'Grösse und Maxillenzahnzahl als Unterscheidungsmerkmal der Rassen von *A. maculipennis* und ihre Beziehungen zur Umwelt'. *Revista Malariologia*, XII, (3), 487-520.

The M. I. of *A. atroparvus* and *A. messeae* is the only constant difference between them, though it is < 1. Variations in size depend on climate and breeding places.

26. Weyer. 'Neuere variationsstatistische Ermittlungen über Flügel-länge und Maxillenindex als Rassenmerkmal von *A. maculipennis*'. *Zool. Anz.*, CIII, Nos. 9-10, 244-253. [Abstract in *Rev. App. Ent.*, (B), XXI, 259.]

The information in this paper is included in No. 25.

1934.

27. Gaschen. 'Récherches entomologiques dans la province du Yunnan'. *Bull. Soc. Med.-Chir. Indo-Chine*, XII, (9), 873-892.

Species.	M. I.
<i>A. sinensis</i> 14 to 16
<i>A. vagus</i> 14 to 16
<i>A. culicifacies</i> 11 to 12
<i>A. minimus</i> 11 to 12

28. Roubaud and Treillard. 'L'indice maxillaire chez *Pseudomyzomyia ludlowi*, (var. *sundaica* et var. *litoralis*) en Indochine méridionale'. *Bull. Soc. Path. Exot.*, XXVII, 552-554.

A 'certain number' counted, at $\times 700$, which neglects the abortive teeth at the extremities. *A. sundaicus* 10 to 12. M. I. 11.5. 20 per cent over this. *litoralis* 11 to 14. M. I. 12.5. 80 per cent over 11.5. Suggests confirmation by more numerous counts.

29. Torres-Canamares. 'Observaciones sobre los *A. maculipennis* y sus razas en Camporredondo (Jaen)'. *Medic. Paises Calid.*, VII, (2), 53-72.

The difference in one pair of maxillæ may be 4. Counts 12 to 19. M. I. 14.9 to 15.0. But here Roubaud found 15.7. Explains by decreased breeding lowering competition. Though M. I. is within Roubaud's safety limits there is severe malaria. M. I. of gorged females 15.1, of ungorged 14.7. The captured females gave eggs attributed to *A. atroparvus*.

30. Toumanoff. 'Quelques faits sur les habitudes trophiques des anophélines d'Extrême-Orient'. *Bull. Soc. Path. Exot.*, XXVII, (10), 932-936.

Paucidentate vectors (and perhaps other paucidentate anophelines) feed on buffaloes rather than on cattle as an alternative to man. 250 *A. tessellatus*, mostly fed on buffaloes, M. I. 11.7. Suggests dogs and pigs as alternative food to man in *A. minimus* and *A. jayporiensis*.

1935.

31. Compagnini. 'Cambiamento dei caratteri somatici della fauna anofelica nella bonifica di S. Eufemia', (2^a Nota). *Revista Malariologia*, XIV, (1), 6, 457.

If only drainage is done M. I. < 14, if agricultural improvements precede drainage M. I. > 14.

Is dealing with a mixture of *A. typicus*, *A. messeae*, *A. labranchiae* and *A. elutus*.

32. De Buen (E.). 'Estudios sobre la biología del *A. maculipennis* Meig., índice maxilar y longitudes de ala, abdomen y torax'. *Medic. Paises Calid.*, VIII, (2), 73-84.

920 spec. examined. M. I. 15.9. If races occur they cannot be distinguished by M. I. or wing-length. The index does not vary with the size of the mosquito, but mosquitoes are smaller in size at higher temperatures. Uses Leitz obj. 6, oc. 8 or 12. Dry obj.

33. Deimer. 'Over biotypen van *A. maculipennis* Mg., in het bijzonder in westelijk Nederland een taxonomisch onderzoek'. A treatise. Review in *Trop. Dis. Bull.*, XXXII, p. 835.

Positively zoophile races are said to have an M. I. of < 14, and the indifferent race of > 14. The distinction is questionable.

34. Galliard and Sautet. 'Nouvelle contribution à l'étude de l'anophélisme en Corse. Les variations saisonnières de l'indice maxillaire'. *Bull. Soc. Path. Exot.*, XXVIII, (6), 453-456.

A. labranchiae and *A. elutus* have an M. I. of 12 to 14 in summer epidemic season, 13 to 16 in winter. This may correlate with ample breeding facilities at the beginning of summer, whereas in September, before first rains, breeding is reduced to a minimum and only robust individuals survive, leading to hibernating individuals with high M. I.

35. Hackett and Missiroli. 'The varieties of *A. maculipennis* and their relation to the distribution of malaria in Europe'. *Revista Malariologia*, XIV, (1), 45-109.

Gives the M. I. of *A. messeae* and *A. atroparvus* from Germany to Central Italy.

36. Ma and Chang. 'Observations on the maxillary teeth of *A. hyrcanus* var. *sinensis* Wd. in Shanghai Region'. *Lingnan J. Sci.*, XIV, 611-615.

M. I. 13 to 21, av. 16.23. 500 pairs examined, 285 equal, others differed by 1 to 5 side to side. M. I. is higher than for same species in North Indo-China.

37. Ottolenghi and Rosa. 'Ricerche sulle varietà di *A. maculipennis* del Ferrarese e di alcune zone limitrofe'. *Revista Malariologia*, XIV, (4), 297-324.

A. atroparvus has an M. I. of 14.5 to 15.0 or higher. *A. typicus*, *A. messeae* and *A. elutus* have an M. I. of 13.5 to 14.0.

38. Rivera and Hill. 'Persistencia de los caracteres diferenciales de los huevos, larvas y adultos, en diferentes generaciones de *Anopheles maculipennis* (*atroparvus*)'. *Medic. Paisés Calid.*, VIII, (7), 313-319.

Breeding experiments (numbers not stated) show M. I. reducing from 16.5 in parent to 14.3 in 7th generation.

39. Roubaud. 'Variété nouvelle de l'*A. maculipennis* au Maroc. *A. m. sicaulti*, nov. var.'. *Bull. Soc. Path. Exot.*, XXVIII, 107.

Distinguishes the new variety on egg characters. M. I. 13.7. Is androphilic by preference.

40. Roubaud and Toumanoff. 'L'indice maxillaire et l'orientation trophique chez les Anophélines d'Extrême-Orient'. *Bull. Soc. Path. Exot.*, XXVIII, (9), 835-838.

Though high M. I. means zoophily and low M. I. androphily and carrying, there are also zoophilic spp. with low M. I. Explain by stating that the process of selection only operates under stress of competition, when many mosquitoes try to feed on a relatively small number of animals, in an enclosed space (entophily). Such is *A. maculipennis* and to a lesser degree Oriental *A. hyrcanus*. These factors do not operate on species, like *bifurcatus*, that feed on animals in the open (exophily). These are wild spp., feeding on wild animals or domestic animals living out of doors, and there is not the same degree of competition. In Indo-China the paucidentate zoophilic spp. are essentially those that attack animals in the open or in inadequate shelters. But *A. minimus* is entophilic and anthropophilic, but man being more numerous and more easily accessible than animals, it has remained paucidentate, like other species that habitually attack man indoors. *A. maculatus*, *A. splendidus*, *A. philippinensis* and *A. kochi*, examined for M. I. and by precipitin test are exceptions to the accepted rule, having a low M. I. (12.5) but are zoophile. But they are exophiles. *A. vagus* is an amphophile. It attacks animals rather than man under cover or in the open, but always returns to domestic shelter. Its variable M. I. suggests that it has races, like *ludlowi*.

41. Sergent (Et.) and Trens. 'Premières études sur les races d'*A. maculipennis* en France et en Algérie, (1933)'. *Arch. Inst. Past. Alger.*, XIII, (1), 1-10.

In Algeria *labranchiae* is definitely zoophile, M. I. 14 to 15. It has not become less dangerous in the localities studied, where conditions have long favoured development of zoophily. It is the only race found in malarious regions. In the Limousin (Corrèze), malaria disappeared over 50 years ago. Here *A. atroparvus* has M. I. 16 to 17. In Alsace, no malaria, *A. messeae* has M. I. 15.6.

42. Toumanoff. 'Etude de l'indice maxillaire de Roubaud en tant que methode pratique d'investigation sur les aptitudes trophiques des Especes Anophelinennes d'Extreme-Orient'. *Trans. IXth Cong., F. E. A. T. M.*, II, 37-51.

In technique follows Roubaud. Examines with a No. 7 obj., occ. comp. 6 ($\times 600$ to 650), in alcohol. Earlier results are included in the present enlarged figures:

Species.	W. Indo-China.	S. Indo-China.	Tonkin.	Cochin-China.
<i>A. sinensis</i>	.. 15.3	15.1
<i>A. vagus</i>	.. 14.1	14.4
<i>A. minimus</i>	.. 11.5	11.3
<i>A. aconitus</i>	.. 11.3	11.3
<i>A. jeyporiensis</i>	.. 11.7	12.9
<i>A. maculatus</i>	.. 11.3
<i>A. annularis</i>	11.4	11.3
<i>A. kochi</i>	11.7	..
<i>A. splendidus</i>	11.6	11.8
<i>A. philippinensis</i>	11.4	11.4
<i>A. subpictus</i>	13.0	14.3
<i>A. tessellatus</i>	12.1	12.4
<i>A. sundaicus</i>	13.7

Work on σ size: M. I. *A. barbirostris* 11 to 17. M. I. 13.3. *A. vagus* 11 to 19, M. I. 14.1. The figures of maxillæ are valuable.

43. Toumanoff. 'Relations entre l'armement maxillaire et l'exploitation relative de l'homme ou des animaux par les espèces anophéliennes indochinoises'. *Bull. Soc. Path. Exot.*, XXVIII, (10), 948-958.

Five-thousand precipitin tests show *A. karwari*, *A. kochi*, *A. splendidus*, *A. tessellatus* and *A. philippinensis* to be zoophilic. A study of the M. I. of *A. sinensis*, *A. barbirostris*, *A. jeyporiensis*, *A. aconitus*, *A. minimus*, *A. sundaicus* and of *A. vagus* with *A. subpictus* showed that in each species the percentage with human blood was highest in individuals with the lowest indices. On the other hand the zoophilic spp., mentioned above, are most often animal engorged, even though the M. I. is usually low, though in some of these spp. there is a high percentage of individuals with M. I. 13 and over. Bovine blood in paucidentate spp. in the delta region may mean buffalo.

44. Treillard. 'Domesticité périodique et périodicité de la pullulation chez les Anophèles extrêmes-orientaux. Remarques sur ses modalités, ses causes et son utilisation'. *Bull. Soc. Path. Exot.*, XXVIII, (6), 448-450.

Zoophilic spp. with high M. I. may be domestic (*A. vagus*). Anthropophilic spp. with low M. I. and power to transmit seem always to be domestic and long-lived.

45. Van Thiel. 'Onderzoekingen omtrent het gedrag van *Anopheles* ten opzichte van mensch en dier, mede in verband met de rassen-studie bij *A. maculipennis*'. *Geneesk. Tijd. Ned.-Ind.*, LXXV, (25), 2101-2118.

Used an immersion lens, and obtained higher numbers than by Roubaud's 1934 method, but uniformly, for all spp. Works out a reduced M. I. for comparison. The red. M. I. of *A. karwari*, *A. annularis*, *A. tessellatus*, *A. maculatus*, *A. aconitus* and *A. kochi* is about 11.5, of *A. nigerrimus*, *A. subpictus* and *A. vagus* > 13. *A. barbirostris* is near the pauci- and *A. sundaicus* near the multidentate group. Yet the latter is the most anthropophilic species in Nederlands-India. *A. nigerrimus*, nearly as anthropophilic, is multidentate.

A few *punctulatus* had an M. I. of 19, the highest of all, yet it is a dangerous vector, whilst *A. tessellatus*, M. I. 11.7, is the least anthropophilic. *A. vagus* is the most multidentate and least anthropophilic sp. in Nederlands-India. He concludes that for that region M. I. is an unreliable guide. The percentage engorged with human blood is: *A. sundanicus* 86, *A. nigerrimus* 83, *A. aconitus* 12, *A. subpictus* 12, *A. maculatus* 9, *A. barbirostris* 9, *A. annularis* 9, *A. kochi* 4, *A. vagus* 1, *A. tessellatus* 0. *A. sinensis* has a lower M. I. than *A. nigerrimus*.

46. Wanson. 'Influences de la salinité sur la faune culicidienne'. *Ann. Soc. Belg. Trop. Med.*, XV, (4), 587-598.

A. gambiae has two wing-length types, but both have an identical M. I. of 12.24.

SUMMARY AND CONCLUSIONS.

1. A summary, with annotated bibliography, of all published work on the maxillary index of *Anopheles* throughout the world is given. It is shown that evidence continues to accumulate that the original hypothesis of Roubaud is well founded. The Oriental species of the genus are shown to conform to values worked out in Europe as regards paucidentate vector species. The Oriental fauna contains no highly multidentate species to test the second part of Roubaud's hypothesis.

2. The Oriental fauna contains several species which are not natural carriers, but are paucidentate, an explanation for which is still to seek. The maxillary index cannot therefore be used to replace dissections in searching for the actual malaria vectors in a fauna.

3. It is shown that the maxillary index of adults bred from larvæ and of adults caught in nature cannot be compared. But the index of adults bred from pupæ within twenty-four hours of emergence has no statistical difference from that of wild-caught adults. Difference in dispersion about the mean is a better test than the mean itself.

4. There is an annual cyclical change in the maxillary index of one species at least that appears to be correlated with rainfall.

5. The maxillary index is constant over wide stretches of country, but there appear to be geographical limits beyond which this is not the case.

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A NOTE ON THE PREVENTION OF HÆMOGLOBINURIA IN
P. KNOWLESI INFECTIONS IN *S. RHESUS* BY
METHYLENE BLUE AND ITS CURATIVE
VALUE WHEN COMBINED WITH
QUININE SALTS.

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[20th April, 1937.]

THE use of methylene blue is not new in the treatment of malaria. Gutmann and Ehrlich (1891) noted its specific effect on malaria and since then methylene blue has been used by numerous workers, mostly in combination with quinine. Although it kills the plasmodia in very high dilutions *in vitro*, methylene blue is less effective for the treatment of malaria as compared to quinine. Anschuetz (1910) found it ineffective in bird malaria, and Pitschugin (1925) claimed that 0.01 gm. for every year of age three times daily for a week cured benign tertian infections. Couto (1926) believed that methylene blue was as efficient as quinine in the treatment of simple tertian infections but not so in malignant tertian cases.

The use of methylene blue for the prevention of the formation of methæmoglobin or other toxic effects produced by the administration of

plasmoquine was suggested to us by Lieut.-Colonel J. A. Sinton, I.M.S. Experiments were, therefore, undertaken to test the efficiency of such treatment in monkeys to which plasmoquine had been administered. In these animals, it was not possible to reproduce the toxic effects noted in human beings, but it was observed in the control animals that the use of methylene blue prevented the occurrence of hæmoglobinuria in *rhesus* monkeys infected with *P. knowlesi* and, when administered as a therapeutic agent along with quinine, acted more efficiently than quinine alone.

Owing to the transfer of one of us (R. C. W.) these experiments were not completed, but a summary of the incomplete work is given below in the hope that it may provide a stimulus for further work to corroborate our preliminary findings.

P. KNOWLESI INFECTIONS IN *S. RHESUS*.

Plasmodial infections of monkeys seem to be eminently suitable for testing the effects and utility of antimalarial drugs, especially *Plasmodium knowlesi* which, when inoculated into *S. rhesus*, almost invariably leads to the death of the animal, and is often accompanied by hæmoglobinuria in the terminal stages of the infection. Mulligan and Sinton (1933), referring to *P. knowlesi* infections in *S. rhesus*, state that 'out of about 120 infections, it has been found that death resulted in every case when treatment of the initial attack was withheld'. More recent data (unpublished) from the Malaria Survey of India, Kasauli, show that out of 254 specimens of *S. rhesus* infected with *P. knowlesi* and from which treatment was withheld, only four escaped death and in the majority hæmoglobinuria was present. If the treatment is not commenced in the early stages of infection, death results in spite of treatment. Recovery takes place only exceptionally when treatment is begun on the third or fourth day of the attack.

MATERIAL AND METHODS USED.

The species of monkey used in these experiments was *S. rhesus*.

The infection in all monkeys used in the experiments was induced by inoculation of blood from another monkey, known to be infected with *P. knowlesi*. About 0.5 c.c. of the infective blood was inoculated intraperitoneally.

Daily examinations of thick and thin smears of the blood of all monkeys under experiment were carried out during the earlier stages of the infection, but after the suppression of the acute attack blood examinations were made only once weekly.

THE USE OF QUININE SULPHATE ALONE AND IN COMBINATION WITH METHYLENE BLUE IN SOLUTION.

In our first observation two monkeys were inoculated with *Plasmodium knowlesi*. In each case parasites appeared on the seventh day and on the third day after their appearance about 70 per cent of the red blood corpuscles were infected. At this stage treatment with quinine mixture (orally) was commenced in both cases and in one case, in addition to quinine, 5 c.c. of 1 per cent methylene blue solution in normal saline were given intravenously. These

observations were repeated in two additional monkeys. The history of these animals is as given below :—

Date of treatment.	Monkey receiving quinine only.	Monkey receiving quinine and methylenic blue.
FIRST OBSERVATION.		
9-8-34	Quinine sulphate 2 grains in solution administered orally.	Quinine sulphate 2 grains in solution orally, and 5 c.c. of 1 per cent methylene blue solution intravenously.
	In the afternoon urine with abundant deposit; hæmoglobinuria present.	Urine almost clear; colour blue due to methylene blue; faint hæmoglobin bands spectroscopically.
10-8-34	Two grains of quinine sulphate orally in solution.	Two grains of quinine sulphate orally in solution and 5 c.c. of 1 per cent methylene blue solution intravenously.
	Hæmoglobinuria persisting; monkey died at 4-35 p.m.	No hæmoglobinuria; monkey remained free from malaria for four months.

SECOND OBSERVATION.

18-1-37	Inoculated with K ₁ strain of <i>P. knowlesi</i> .	Inoculated with K ₁ strain of <i>P. knowlesi</i> .
26-1-37	Showed infection in about 60 per cent of erythrocytes.	Showed infection in about 60 per cent of erythrocytes.
	Two grains of quinine sulphate given orally in mixture.	Two grains of quinine sulphate in solution given orally, and 5 c.c. of 1 per cent methylene blue solution intravenously.
		No hæmoglobinuria.
27-1-37	Dark brown urine; hæmoglobinuria present; condition of the animal poor. Two grains of quinine sulphate repeated.	Urine tinged blue, faint bands of hæmoglobin spectroscopically. Two grains of quinine sulphate repeated.
29-1-37	Scanty parasites; no hæmoglobinuria.	Parasites absent; no hæmoglobinuria or blue colour of urine.
30-1-37	Scanty parasites; no hæmoglobinuria; animal died in the evening.	Parasites absent; no hæmoglobinuria. This monkey died a fortnight later from dysentery; no parasites detected in blood smears from organs at autopsy.

THE USE OF METHYLENE BLUE ALONE AND IN COMBINATION WITH QUININE SULPHATE.

(i) ADMINISTRATION OF METHYLENE BLUE ALONE.

- 26-11-34. Monkey inoculated with *P. knowlesi*.
 4-12-34. Infection detected in blood smears.

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7-12-34. About 70 per cent of the erythrocytes infected with the parasites.

Hæmoglobinuria present. 5 c.c. of 1 per cent methylene blue solution administered intravenously.

8-12-34. Trophozoites, gametocytes, etc., seen in the blood smears; monkey acutely ill; no hæmoglobinuria; monkey died in the afternoon.

(ii) ADMINISTRATION OF METHYLENE BLUE IN COMBINATION WITH QUININE SULPHATE.

9-10-34. Monkey inoculated with *P. knowlesi*.

29-10-34. About 70 per cent of the erythrocytes infected; 5 c.c. of 1 per cent methylene blue solution given intravenously and 2 grains of quinine sulphate in solution by mouth; hæmoglobinuria present.

30-10-34. Parasites scanty; no hæmoglobinuria; no further treatment given.

The animal recovered, but relapsed after a fortnight. Then the above treatment was repeated and resulted in suppression of the infection (subsequently observed for three months).

DISCUSSION.

The development of hæmoglobinuria has been variable with different strains of *P. knowlesi*. It has been noted that, when infected with certain strains of *P. knowlesi*, a large number of animals may die without developing hæmoglobinuria. Our experiments, though too few to draw any definite conclusions, seem to suggest that the administration of quinine sulphate when the infection is at its height, either brings about or expedites the onset of hæmoglobinuria. In certain cases of human malaria the 'quinine hæmoglobinuria' is too well known to be discussed here. The work of Nocht and Kikuth (1929) has shown that quinine salts *in vivo* undoubtedly act as excitants to hæmolysis and hæmoglobinuria when given with small doses of hæmolytic substances which in themselves are insufficient to produce either. Many blame the administration of quinine for bringing about the onset of blackwater fever in certain hyper-endemic areas. We feel that the administration of methylene blue given simultaneously with quinine salts is worth a trial in such cases as it would check any tendency of the latter to produce hæmoglobinuria and at the same time enhance its antimalarial value considerably.

SUMMARY.

A small series of *rhesus* monkeys infected with *P. knowlesi* was treated with quinine sulphate alone, with methylene blue alone, and with a combination of these two drugs. The following inferences seem to be justified from these experiments :—

1. When the infection is at its height, quinine administered alone may expedite the onset of hæmoglobinuria which may or may not be present in untreated cases.

2. The administration of methylene blue alone prevents the development of hæmoglobinuria in monkeys but has no effect on plasmodia. When the latter

drug is given simultaneously with quinine sulphate it cures *P. knowlesi* infection in *S. rhesus*, this combination of drugs being more potent than quinine alone.

ACKNOWLEDGMENTS.

We wish to record our thanks to Lieut.-Colonel J. A. Sinton, v.c., o.b.e., m.d., d.sc., d.p.h., d.t.m., i.m.s., formerly Director, Malaria Survey of India, Kasauli, and to Lieut.-Colonel S. S. Sokhey, m.a., b.sc., m.d., d.t.m. & h., i.m.s., Director, Haffkine Institute, Bombay, for the facilities afforded us to carry out the work.

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PARIS GREEN AND PADDY*.

BY

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[26th April, 1937.]

IN 1935 Covell published an article summarising the existing literature, and giving opinions of various workers, on the effects of paris green dusting on rice crops. His conclusions were ' (1) The general opinion amongst malaria workers who have had experience of paris green dusting of rice fields in various countries is that, in the quantities in which it is applied for antilarval purposes, this larvicide exerts no harmful effect on the rice crop. (2) There is some evidence, however, that harm may result from dusting paris green over the open flowers of the rice plants. It is recommended that during the flowering season the application should be restricted to the afternoon, i.e., during the period when the flowers are closed '.

The office of the Nagenhalli Station for the Study and Control of Malaria of the Mysore State Department of Health is located in the buildings of an experimental paddy farm of the Department of Agriculture. Advantage was, therefore, taken of this arrangement to study the effect of paris green dusting on the yield of paddy crops in grain, straw, and chaff. Observations were commenced in 1934 and were continued on the crops of 1935 and 1936, similar areas of growing paddy being used for experiment and comparison plots. The writers of this report wish to express their thanks to the officers of the Mysore State Department of Agriculture who provided the facilities and carefully measured the yields of these plots.

All the paddy under test was of the same variety and was grown under the same water, manurial, and other conditions, each year entirely comparable plots being selected for dusting with paris green and for comparison without dusting. The experiments of the various years were as follows :—

1934.—Fifteen paddy plots, totalling 0.647 of an acre, were dusted with a one per cent paris green mixture, dusting beginning after the flowering of the paddy; comparison plots, not dusted, also numbered 15 and covered 0.637 of an acre.

* The work here reported was done under the auspices of the Department of Health, Government of Mysore, and the International Health Division of the Rockefeller Foundation.

1935.—Five paddy blocks, totalling 0.719 of an acre, were selected for dusting and five plots comprising 0.848 of an acre, for comparison. The paddy was transplanted on the 23rd and the 24th August, paris greening began on the 25th September, flowering came on the 25th October, dusting stopped on the 11th December, and the paddy was harvested on the 18th December. Paris green dusting was continued throughout the flowering season.

1936.—Paddy was transplanted into nine plots on the 15th and the 16th of July 1936, and paris green dusting began on six plots on the 29th July. (A) The paris green dusting was continued throughout the growing period on three plots and was stopped on the 18th November, just before harvesting. (B) On another three plots dusting began on the same date but was stopped entirely between the 14th October and the 4th November, when the paddy was in flower; it was then resumed and ceased on the same day as for the plots of (A). The remaining three plots receiving no paris green served as a comparison for the two sets of dusted plots.

All the paris green dusting was done in the morning, once weekly, with a one per cent paris green mixture in 55 to 44 road dust and wood ash. It was applied by ordinary hand blowers and in quantities usually used for larval control in this area.

The grain yields, with their standard deviations, of the dusted and comparison plots for all the years and for yearly experiments are given in pounds per acre in Table I and the complete reports of yields of grain, straw, and chaff of individual plots are given in the Appendix.

TABLE I.

Grain yields in pounds per acre of paris green dusted and comparison paddy plots.

Period of paris green dusting.	PARIS GREEN DUSTED PADDY PLOTS.		COMPARISON PLOTS NOT DUSTED.	
	Mean.	Standard deviation.	Mean.	Standard deviation.
All plots, all years	2,606	645	2,467	347
1934. Paris green dusting begun after flowering season.	2,742	751	2,459	349
1935. Paris green dusting continued throughout growing season.	2,020	180	2,227	73
1936 (A). Paris green dusting continued throughout growing season.	2,743	143	2,908	105
1936 (B). Paris green dusting stopped during flowering season.	2,764	52		

For all of the paddy plots on which paris green was dusted during the three years the mean yield of rice grain was 2,606 pounds per acre, with a standard deviation of 645 pounds, and for comparative plots not dusted the yield was 2,467 pounds with a standard deviation of 347 pounds per acre. The difference between these two yields was 139 pounds which is less than the standard deviation of the mean yield of the comparison plots.

In 1934 when paris green dusting began after the flowering of the paddy, the difference between the two mean yields of rice was 283 pounds which is again less than the standard deviation of 349 pounds per acre of the mean of the corresponding comparison plots.

The standard deviations of the mean yields of grain for the plots dusted with paris green in all years and in 1934 were both very much larger than the standard deviations of the corresponding comparison means. This was entirely due to one small plot of 0.29 of an acre, dusted with paris green in 1934, which produced the very high yield of 5,241 pounds of rice per acre, about twice the usual yield. Removing this one plot from consideration reduced the mean yield, and its standard deviation, for all years, to 2,500 pounds and 379 pounds and for 1934, to 2,563 pounds and 356 pounds per acre.

In 1935 the average yield of grain in plots dusted with paris green throughout the growing season was 2,020 pounds per acre, with a standard deviation of 180 pounds, as against a mean yield in comparable undusted plots of 2,227 pounds, the standard deviation being 73 pounds per acre. The difference between these two yields was 207 pounds which was 2.83 times the standard deviation of the mean of the comparison plots but only 1.15 times the standard deviation of the mean of the dusted plots. The chances against a variation as great as 2.83 times the standard deviation are approximately 95.5 in 100, but such variations may occur very rarely. The difference between the two means being only 1.15 times the standard deviation of the means of the dusted plots is rather in favour of the assumption that this variation was a chance one and not due to the paris green dusting since there is no evidence in the other results that the use of paris green increases the variability of yields of rice (when the exceptional increase of yield of plot 13 of 1934 is not included). The experiment was repeated in 1936 (A), when the corresponding difference between the dusted and comparison yields was 165 pounds per acre, only 1.57 times the standard deviation of the mean of the comparison plots and well within the bounds set by chance. When the dusted and comparison plots of 1935 were combined with those of 1936 (A), the average yields and standard deviations were 2,291 and 388 as compared to 2,482 and 341 pounds per acre. The difference here was 191 pounds which is well below either standard deviation. The fact that the average yields of rice in both the dusted and undusted plots were more than three times their standard deviations below the average yields of the other years (which did not differ from each other) would seem to indicate that some factor other than paris green was operating in 1935 to decrease the yield of rice in some unexplained way. On the whole it seems fair to conclude that dusting with paris green throughout the growing season did not affect the yield of rice.

When paris green dusting in 1936 was stopped during the flowering of the paddy [1936 (B)], the mean yield was 2,764 pounds per acre with a standard deviation of 52 pounds as against a mean yield in comparable undusted plots

of 2,908 pounds, the standard deviation being 105 pounds. The difference between the means was 144 pounds which was 1.37 times the standard deviation of the comparison mean and well within a chance variation. Further, the mean of 2,764 pounds when dusting was stopped during flowering was not significantly different from the mean of 2,743 pounds when dusting continued throughout the 1936 season. It is of interest to note, also, that the mean yield of grain in the 1934 dusted plots, when dusting began after flowering, was not different from the average of the 1936 plots dusted throughout the growing season.

When the average yields of straw and chaff, with their standard deviations, were computed (*vide* Table II), no evidence could be found of any effect of paris green dusting in varying the yields significantly, all differences between the dusted and undusted average yields being less than twice the standard deviation of the comparison means.

TABLE II.

Yields in pounds per acre of straw and chaff from paddy plots dusted, with paris green and undusted comparison plots.*

Year.	YIELD OF STRAW.				YIELD OF CHAFF.			
	DUSTED PLOTS.		COMPARISON PLOTS.		DUSTED PLOTS.		COMPARISON PLOTS.	
	Mean.	Standard deviation.	Mean.	Standard deviation.	Mean.	Standard deviation.	Mean.	Standard deviation.
All years ..	3,949	1,030	4,254	1,034	113	27	100	35
1934 ..	4,558	851	4,787	771
1935 ..	2,662	527	2,844	419	107	37	77	16
1936 (A) ..	3,476	276	3,939	309	120	8	138	24
1936 (B) ..	3,524	165			115	11		

* See text and Table I for details of paris green dusting.

SUMMARY.

In a series of paddy plots dusted with a one per cent dilution of paris green in road dust and wood ash, in quantities usually used for larval control in Mysore, no effects could be demonstrated which indicated that paris green decreased the rice and straw yield or increased the amount of chaff. There was no indication that stopping paris green dusting during the period in which the paddy was in flower was necessary.

REFERENCE.

COVELL, G. (1935) The Effect of Paris Green Dusting on Rice Crops.
Rec. Mal. Surv. Ind., 5, 2, pp. 153-157.

APPENDIX.

Yields in pounds per acre of grain, straw, and chaff from paris green dusted and comparison undusted paddy plots for experiments of three years.

Year.	No.	PARIS GREEN DUSTED PLOTS.			COMPARISON UNDUSTED PLOTS.		
		Grain.	Straw.	Chaff *.	Grain.	Straw.	Chaff *.
1934 ..	1	2,390	2,780	..	1,519	4,222	..
	2	2,528	4,917	..	2,692	5,077	..
	3	2,722	5,111	..	2,719	5,281	..
	4	3,055	4,145	..	2,536	4,857	..
	5	2,280	4,220	..	2,467	4,022	..
	6	2,277	4,255	..	2,180	3,852	..
	7	2,425	4,400	..	2,275	4,137	..
	8	3,300	3,900	..	2,316	4,211	..
	9	2,679	4,226	..	2,123	3,809	..
	10	1,878	3,429	..	2,414	4,586	..
	11	2,911	5,022	..	2,917	6,278	..
	12	2,432	5,136	..	2,829	6,171	..
	13	5,241	6,483	..	2,400	4,629	..
	14	2,750	5,071	..	2,917	5,722	..
	15	2,259	5,278	..	2,574	4,944	..
1935 ..	1	2,319	2,117	67	2,312	3,223	64
	2	1,938	2,338	94	2,237	2,384	53
	3	1,811	2,406	123	2,103	2,941	81
	4	1,911	2,846	171	2,199	2,329	87
	5	2,120	3,605	78	2,284	3,343	98
1936 (A)	1	2,893	3,685	119
	2	2,550	3,085	111
	3	2,787	3,657	130
1936 (B)	1	2,699	3,327	103	2,912	3,541	113
	2	2,768	3,514	113	3,035	3,983	130
	3	2,825	3,730	130	2,777	4,293	170

* Not measured in 1934.

A STUDY OF VILLAGE MALARIA IN MYSORE STATE*.

BY

W. C. SWEET, M.D., DR.P.H.

[11th May, 1937.]

INTRODUCTION.

DURING the year 1935 a study of uncontrolled malaria was made in three villages of the T. Narsipur Taluk of the Mysore District, 16 miles south-east of Mysore City. The villages of Gargeshwari, Yedadore, and Byrapura were chosen for this study, the office and field laboratory being in the first named. Gargeshwari and Yedadore, which are within half a mile of each other, lie on a narrowing tongue of land above the junction of the Cauvery and Kabbani Rivers, both being within a few furlongs of the bank of the Cauvery and about three-quarters of a mile from the left bank of the Kabbani. The whole tongue of land, with the exception of small village reserves, is heavily irrigated from June to January and extensive paddy fields cover the area during these months. Byrapura lies on the right bank of the Kabbani River, within two furlongs of the river and about one mile in a straight line across the river from Gargeshwari. There is no wet cultivation on this side of the river within a mile and a half of Byrapura which is over three-quarters of a mile from the town of T. Narsipur (*vide* Map).

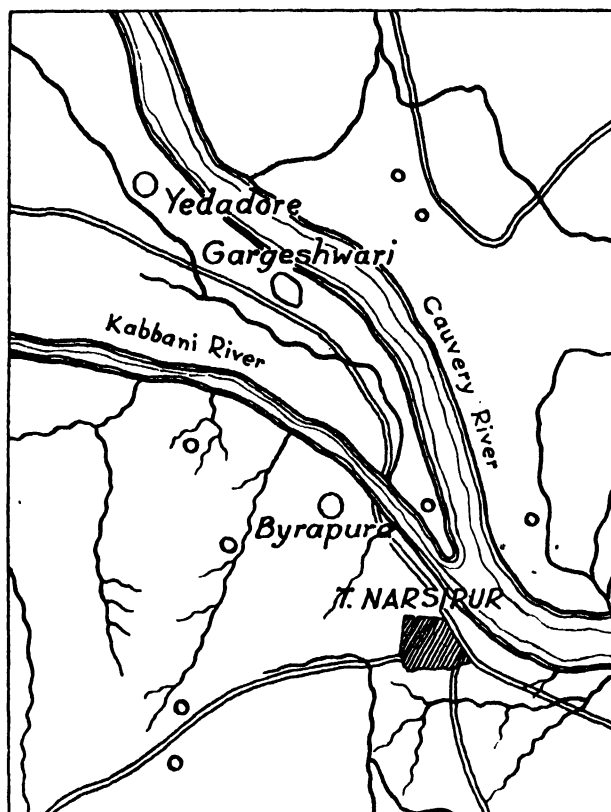
The population of Gargeshwari is 1,846 and that of Yedadore and Byrapura 658 and 563 respectively. Residents of Gargeshwari are mainly landowners and shopkeepers while those of Yedadore are mainly agricultural labourers; Byrapura represents the more usual Mysore village of agriculturists. The populations of all three are static and do not travel much although Gargeshwari is on the main road and relations with other places are therefore more free. The Yedadore labourers work as a rule for the more wealthy Gargeshwari landowners. Relations between Gargeshwari and Yedadore are very close but Byrapura is isolated from these two villages, its relations being mainly with the neighbouring town of T. Narsipur. For the past two years or so a bridge

* The work here reported was done under the auspices of the Government of Mysore and with the support of the International Health Division of the Rockefeller Foundation.

has been in process of construction across the Kabbani River and one of the labourers' camps was situated within two furlongs of the village. These labourers came from other parts of Mysore and the Madras Presidency, in the main apparently from malarious areas.

The whole area is in the neighbourhood of 2,200 feet above mean sea level and, according to records maintained at T. Narsipur, has had an average rainfall, for the last ten years, of 28·64 inches per annum. The rainfall for

Map of Gargeshwari area.



these years and the average per month are given in Table I. No temperature or humidity records are available.

Since 1926, there have been four years in which the annual rainfall was below the ten years' average and five years in which it was above, 1931 being the lowest and 1933 the highest. There are two rainy seasons in the year. In April and May the average rainfall is 8·41 inches and in August, September, October, and November the area receives 15·19 inches of its yearly average. The months of June and July are intermediate with 3·13 inches, leaving but 1·01 inches for the remaining four months.

TABLE I.

*Rainfall in inches for the past ten years and monthly averages for these years.
Records kept at T. Narsipur.*

Year.	Rainfall in inches.	Month.	Average rainfall in inches.
1926	24.04	January	0.14
1927	24.43	February	0.38
1928	31.40	March	0.70
1929	31.81	April	3.08
1930	33.30	May	5.33
1931	22.38	June	1.56
1932	28.87	July	1.57
1933	34.91	August	3.56
1934	30.60	September	3.53
1935	24.68	October	5.31
..	..	November	2.79
..	..	December	0.69
Average	28.64	For average year	28.64

HISTORY OF MALARIA.

There is a dispensary at T. Narsipur to which the residents of these three villages, as well as those of other nearby villages, go for treatment. The total number of persons treated, the number diagnosed as having malaria, and the percentage of malaria cases are given in Table II for the past ten years, as well as the average percentage treated monthly during these years.

TABLE II.

History of malaria cases at T. Narsipur dispensary for the past ten years.

Year.	Total cases attending dispensary.	Number diagnosed as having malaria.	Percentage of malaria cases.	Months.	Percentage of average monthly cases diagnosed as malaria.
1926	8,786	235	2.7	January	13.0
1927	11,455	682	6.0	February	13.1
1928	10,566	816	7.7	March	17.5
1929	12,438	2,224	17.9	April	17.4
1930	11,509	1,113	9.7	May	12.1
1931	13,081	979	7.5	June	10.4
1932	13,382	663	5.0	July	9.4
1933	15,211	786	5.2	August	7.4
1934	18,397	3,134	17.0	September	8.8
1935	22,037	6,040	26.2	October	11.9
				November	13.9
				December	12.0

From a low point in 1926, the percentage of dispensary malaria cases increased year by year to a high point of 17.9 in 1929. There was a decrease subsequent to this with a sudden increase in 1934, just five years after the

previous high point. However, instead of again declining the rate went higher in 1935 to the highest point reached, namely, 26.2 per cent. In view of similar reports from Ceylon, it is of interest to note the lapse of five years between 1929 and 1934.

The average monthly proportion of malaria at the dispensary has a low point of 7.4 per cent in August and shows a gradual increase to high points of 17.5 and 17.4 in March and April, after which it declines. It should be noted that these highest proportions come before the high rainfall of April and May and in the hot months, while the beginning of the rise after August is subsequent to the start of the higher rainfall and cooler weather of the north-east monsoon.

No definite relation between the monthly and yearly dispensary malaria proportions and the rainfall can be determined. The first high malaria figures in 1929 followed a year of more than average rainfall but further increases in rainfall did not prevent a drop; the lowest annual rainfall of 1931 was followed in the next year by the lowest malaria proportion but decreasing rainfall in 1934 and 1935 did not keep down the malaria. On the other hand, the highest average monthly dispensary proportions of malaria cases appear to be subsequent to the months of low rainfall, although they begin to rise after the onset of the north-east monsoon. The high malarial incidence in 1929 was preceded by an excessive rainfall both in October 1928, and in April 1929, but no such rainfall distribution was true of the 1934-35 rise in malaria. It should be remembered that these are purely clinical diagnoses of malaria, usually based on a complaint of 'fever'.

In December 1932, a spleen survey of this area was made by one of the officers of the Mysore State Department of Health. He examined 73 children in Byrapura and found no enlarged spleens and the same condition existed among 246 children examined in T. Narsipur. The spleen index of 95 children examined in Gargeshwari was 5.3 per cent. Unfortunately no examinations were made in Yedadore.

The people of Gargeshwari stated that malaria had not been prevalent among them until 1934 when they evacuated their village on account of plague. At that time they lived for some months in temporary huts erected on vacant land between Gargeshwari and Yedadore and near the latter village. They suffered heavily from malaria there and after return to their village, but stated that the incidence of malaria decreased in 1935.

Yedadore was founded some 12 years ago by former residents of Gargeshwari following a heavy flood which seriously affected the latter. They stated that for the first few years they were free from malaria but that there was an epidemic later (possibly 1929), and that since then they had had malaria constantly. Both 1934 and 1935, however, were worse than usual in this respect.

The residents of Byrapura complained bitterly of malaria during 1935, a condition they said they had not suffered from before.

SPLEEN EXAMINATIONS.

Spleen and blood examinations were made in Gargeshwari in December 1934, and in eight months of 1935; they were made in Yedadore and Byrapura in four months of 1935. The children examined were between 0 and 14 years of age, those between 10 and 14 numbering 464 in Gargeshwari, 67 in Yedadore,

and 55 in Byrapura, while those between 0 and 4 inclusive numbered 33, 71, and 66, respectively. A total of 1,221 spleen examinations was made in Gargeshwari, 357 in Yedadore, and 293 in Byrapura, to a considerable extent the same children being examined each time. The spleen indices for all months of children 0 to 9 years of age were not different in any of the three places from the indices for the 10 to 14-year children, so Table III gives these indices by months in which examinations were made for all children examined. The spleen index for Gargeshwari was 21.0 per cent, for Yedadore it was 73.7, and for Byrapura 15.0. The Yedadore index was higher than those for either of the other two areas consistently in all months in which examinations were made, but while Byrapura had a lower index than Gargeshwari in February and April and also on the average of all examinations, there was no appreciable difference in the indices of the two places for July and October (*vide* Table III).

In Gargeshwari there was an increase in the spleen index between January and April, with the highest index of 27.9 per cent in the latter month. Yedadore had a uniformly high splenic index with a high point of 81.7 in October, an index considerably greater than the lowest one of 66.7 in July. Byrapura had an index of only 9.0 per cent in February, a figure which is usually understood to indicate an area of low malarial endemicity; this index

TABLE III.

Spleen indices of the Gargeshwari area. Children aged 0 to 14 years.

Months.	GARGESHWARI.			YEDADORE.			BYRAPURA.		
	Number examined.	Number with enlarged spleen.	Spleen index, per cent.	Number examined.	Number with enlarged spleen.	Spleen index, per cent.	Number examined.	Number with enlarged spleen.	Spleen index, per cent.
1934 December	54	9	16.7
1935 January ..	184	27	14.7
February ..	139	34	24.5	79	55	69.6	78	7	9.0
March ..	137	33	24.1
April ..	116	33	27.9	101	76	75.2	89	12	13.5
May ..	136	27	19.9
July ..	151	29	19.2	84	56	66.7	51	7	13.7
August ..	158	33	20.9
October ..	146	31	21.2	93	76	81.7	75	18	24.0
All months	1,221	256	21.0	357	263	73.7	293	44	15.0

had increased to 24.0 per cent by October, indicating a mild epidemic in this village.

Spleen sizes were recorded in seven classes, the smallest being a 'P' spleen palpable just under or at the costal margin on inspiration; classes 1 to 3 were between the costal margin and the level of the umbilicus and classes 5 and 6 below this level; no class 6 spleens were recorded. The spleen sizes found in all examinations are given in Table IV.

The application of the χ^2 test to these distributions of spleen sizes shows that the samples from Gargeshwari and Yedadore are not random samples from the same universe, and therefore that Yedadore had a significantly greater proportion of large spleens than did Gargeshwari. The same was true when Yedadore and Byrapura were considered, the former having the greater proportion of large spleens. No difference could be established between the spleen size distributions of Gargeshwari and Byrapura. The respective χ^2 for the three pairs of distributions mentioned were 83.3, 24.0 and 1.1.

TABLE IV.
Sizes of enlarged spleens in Gargeshwari area.

Village.	Spleens, size P.	Spleens, size 1.	Spleens, size 2.	Spleens, size 3.	Spleens, sizes 4 and 5.	Total enlarged spleens.
Gargeshwari ..	129	111	13	2	1	256
Yedadore ..	40	161	51	9	2	263
Byrapura ..	20	21	3	0	0	44

MALARIA PARASITES.

Blood slides were taken from each child at the time of the spleen examination and from four additional children in Gargeshwari. The parasite index for all examinations was 6.9 per cent in Gargeshwari, 41.7 per cent in Yedadore, and 16.4 per cent in Byrapura. The differences between these indices are marked and the malaria, as represented by the parasite index, may be taken to be in proportion to the figures given. The monthly parasite indices of the three places and for all ages are given in Table V. In these examinations it was found that the children from 0 to 9 years in Gargeshwari had a parasite index of 8.7 while those from 10 to 14 years had an index of 3.9 per cent, but no difference could be demonstrated between these two age groups in the villages of Yedadore and Byrapura.

There was a high point in the parasite index in Gargeshwari in December 1934, and a secondary rise in August 1935. However, with the exception of the possibly significant fall in index between December 1934, and April 1935, no marked changes occurred in the parasite index from month to month through the year 1935. This was in contrast to the spleen indices in which the April level was well above that of January.

Yedadore had uniformly high parasite indices, the lowest being 30.7 in April and the highest 62.3 per cent in October. The October index was

TABLE V.

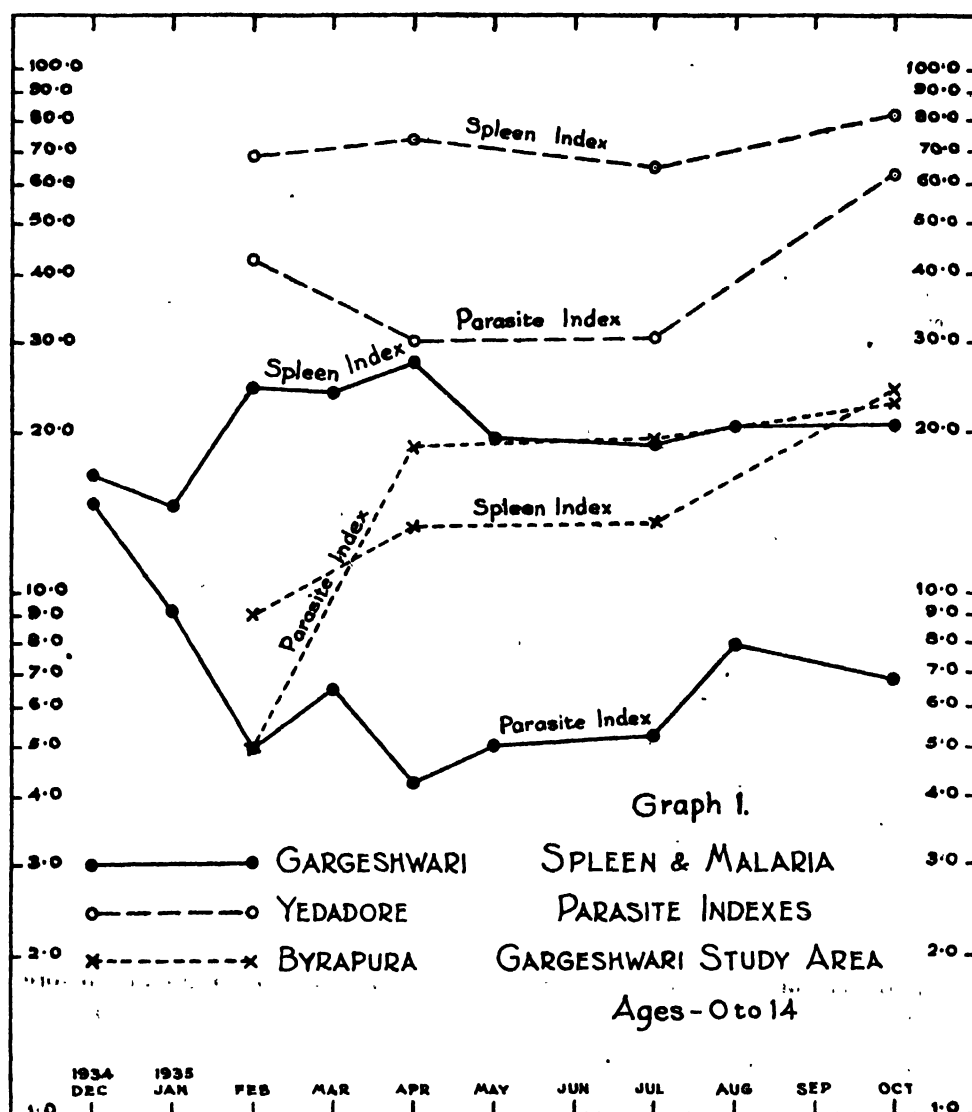
Malaria parasites found in blood examinations, by months. Ages 0 to 14 years.

Months.	GARGESHWARI.			YEDADORE.			BYRAPURA.		
	Number of persons examined.	Number showing malaria parasites.	Parasite index, per cent.	Number examined.	Number infected.	Parasite index, per cent.	Number examined.	Number infected.	Parasite index, per cent.
1934 December	54	8	14.8
1935 January ..	184	17	9.2
February ..	139	7	5.0	79	34	43.0	78	4	5.1
March ..	137	9	6.6	101	31	30.7	89	17	19.1
April ..	116	5	4.3
May ..	136	7	5.1
July ..	151	8	5.3	84	26	30.9	51	10	19.6
August ..	162	13	8.0
October ..	146	10	6.8	93	58	62.3	75	17	22.7
All months	1,225	84	6.9	357	149	41.7	293	48	16.4

considerably higher than that of the other three months but the indices for February, April and July were probably not different. This corresponds to changes in the spleen indices.

The February parasite index of Byrapura was 5.1 which was the same as the 5.0 per cent of Gargeshwari in the same month. The spleen index for this month was 9.0 with which one would not expect to find a parasite index of the level found; the 5.1 parasite figure more nearly corresponds to the 24.5 spleen index which Gargeshwari had in this month. There was a marked increase in the parasite index by April and a slow rise continued until a maximum of 22.7 per cent was reached in October. It would seem that Byrapura had an epidemic of malaria, the rise in infection beginning before February as the parasite index of that month was out of proportion to the spleen index. The parasite index was numerically higher than the spleen index and remained so until October when the figures were 22.7 and 24.0 respectively, still out of proportion. This is what might be expected theoretically in an epidemic of malaria in a population previously free of regular infection. It is possible that, had examinations been made for very small spleens well up under the costal margin, these relations might have been reversed. All the indications point to an epidemic condition in the village of Byrapura which was previously relatively free from malaria.

The three species of parasites were found in the area as a whole in the following proportions: *P. vivax*—31; *P. falciparum*—58; *P. malariae*—11. The corresponding figures for the Nagenhalli area, about 18 miles away, were 46—11—43 (Sweet, 1933). Both Gargeshwari and Yedadore had larger proportions of *P. falciparum* infections than Byrapura and the latter had a



greater proportion of *P. vivax* than Gargeshwari. There were no marked differences between the three places in the *P. malariae* proportions, and Gargeshwari and Yedadore were not different in their ratios of any species of malaria parasite.

Infections with more than one species of parasite were most common in Yedadore (7.6 per cent), next most common in Byrapura (2.7 per cent), and least in Gargeshwari (0.5 per cent). The multiple infection index was smallest in Gargeshwari and higher in Yedadore than in Byrapura. It is of interest to note that this index was highest in October in Yedadore and that no multiple infections were found in Byrapura in February but were present in the other three months. The monthly indices were never significant in Gargeshwari but one or two multiple infections were found there in March, May, July, and October.

To summarise, this was a small rural area in which three quite different conditions in regard to malaria were found. Gargeshwari village had a moderate spleen index, which showed some seasonal increase, and a low parasite index which showed no significant variations in 1935. Judging from the histories given, 1934 was a year with a mild epidemic, so it seems probable that the normal spleen and parasite indices of Gargeshwari are even lower than those of 1935, but it is also probable that it is never entirely free from malaria (*vide* 1932 figures).

Yedadore presented quite a different picture. Here the spleen and parasite indices were those of a high degree of endemic malaria with added increased seasonal prevalence. This increase had also probably been present in 1934 and both indices in 1935 may have been higher than usual in consequence. In spite of this, however, the conclusion is forced that malaria is endemic in this village.

From the spleen and parasite indices in February 1935, and the histories given, it would seem that Byrapura is, as a rule, practically free from malaria, a condition which was completely changed in 1935 by a mild epidemic. The neighbouring town of T. Narsipur, with a zero splenic index in 1932, had an index among 258 children (0 to 14 years) of 14.0 ± 1.5 in July 1935, but it seemed more likely that the source of the parasites of the epidemic in 1935 was the camp of bridge labourers within a furlong or two of Byrapura. Apparently Byrapura did not share in the 1934 increase in malaria to any extent. (Graph 1 gives the spleen and parasite indices for 1935 for the three villages.)

CATCHES OF DANGEROUS ANOPHELES.

Eight buildings in Gargeshwari village were selected as permanent stations for the capture of resting anophelines in the daytime and the same number was selected in each of the other two villages. Each station was visited once a week and catches were made in the morning for twenty minutes in each place. The average catches per station per week made in the daytime resting places of dangerous female anophelines are given by months in Table VI. The dangerous anophelines were considered to be *A. culicifacies*, *A. fluviatilis*, *A. stephensi*, and *A. varuna*. *A. culicifacies* constituted 97.6 per cent of the total catch in Gargeshwari, 95.0 per cent in Yedadore, and 98.7 per cent in Byrapura; the respective percentages of *A. fluviatilis* were 1.2, 4.4, and 0.3, and those of *A. stephensi* were 1.1, 0.4, and 0.9. *A. varuna* was a rare species throughout.

The catches of dangerous female anophelines were approximately equal in Gargeshwari and Yedadore. In Byrapura they were markedly below the

catches in the other two villages from April to September inclusive, about equal in February and October, and above the Gargeshwari and Yedadore catches in March, November, and December. There may be some question as to whether such catches represent in any way the actual density in nature of the species caught. Although it would not seem to be possible to deny that they do represent the general trend of the density throughout a time period in any single area, their application for deductions as to density in neighbouring areas may be questioned. However, in a recent article Barber (1936) says, 'the averages, based on the number of houses or stables visited, are only approximations, since it was impracticable to collect all of the anophelines in a given room; but the figures have some value in the comparison of the density of different species as they occur in villages in July'. It would also seem to be of interest in this connection to note that the average catches of dangerous female anophelines in Mysore were significantly correlated with the parasite rates, in three study areas previously reported, when a lag of one month was used for the parasite rates (Sweet, 1933). The small number of observations in the Gargeshwari area prevented the application of this test to the catches here reported.

TABLE VI.

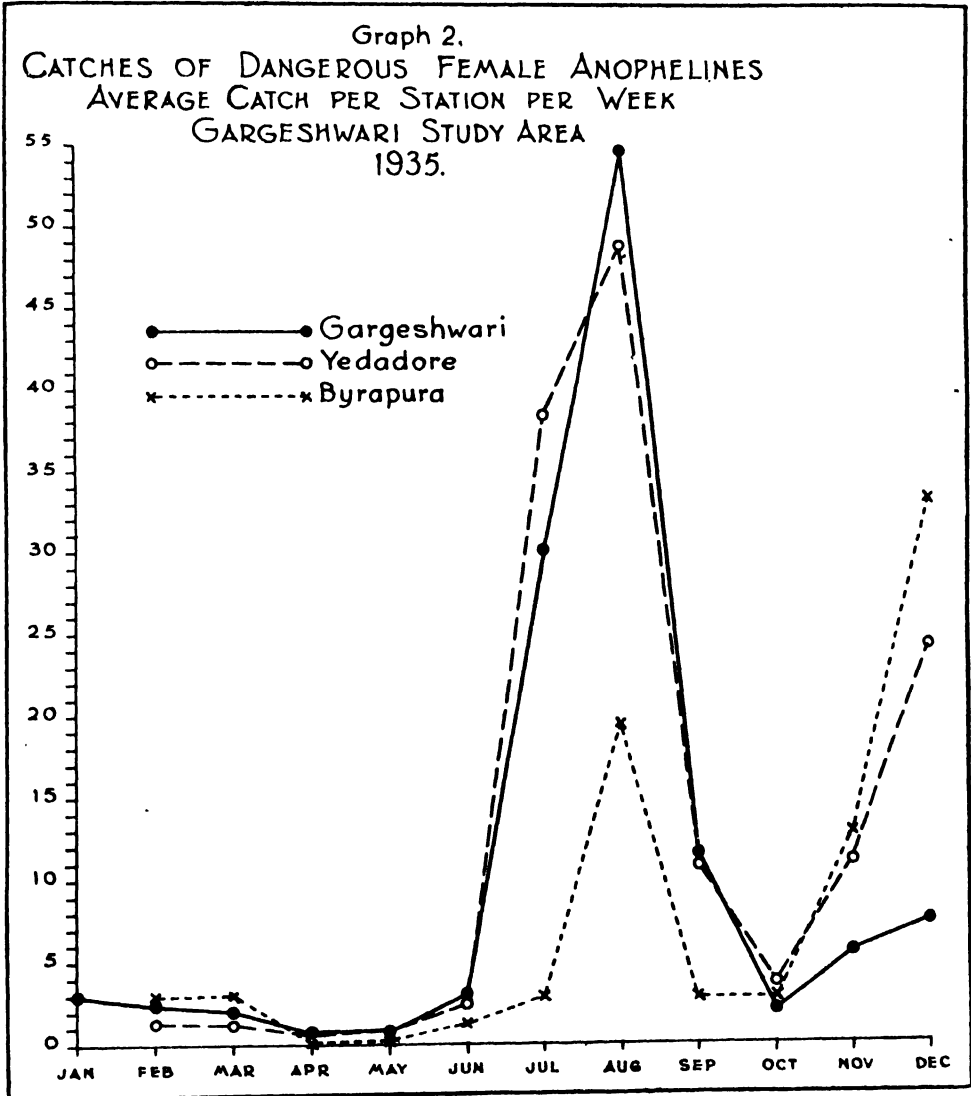
Average catch per station per week made in daytime resting places of dangerous female anophelines.

Months, 1935.	Gargeshwari village.	Yedadore sub-area.	Byrapura sub-area.
January ..	3.0	..	2.9
February ..	2.4	1.3	..
March ..	2.1	1.2	3.0
April ..	0.7	0.6	0.1
May ..	0.8	0.8	0.2
June ..	3.0	2.4	1.2
July ..	30.2	38.5	2.8
August ..	54.7	48.9	19.6
September ..	11.6	10.8	2.8
October ..	2.0	3.7	2.8
November ..	5.5	11.2	12.9
December ..	7.5	24.4	35.4

A study of Table VI demonstrates that there was an actual density of dangerous anophelines in Byrapura sufficient to transmit malaria actively. With some reservations then, as to the application of the catch figures, it may be supposed that the actual densities in Gargeshwari and Yedadore were considerably above the required density. Why did a spread of infections occur in Yedadore but not in Gargeshwari? (*vide* Graph 2).

Due to the heavy catches of *A. culicifacies* the remarks of the last paragraph apply mainly to that species. Previous work in Mysore State was reported (Nursing, Rao, and Sweet, 1934) as showing that catches made in stations in the daytime would not truly represent the effective density of *A. fluviatilis*, this being true in two other areas but not so marked in Nagenhalli, some 18 miles from Gargeshwari. However, larval catches of *A. fluviatilis* were

always low in the Gargeshwari area and no larvæ at all were found in March, April, and May, months in which Byrapura increased its parasite index greatly; there was never any indication in larval findings to suggest that *A. fluviatilis* was a hidden factor in malaria transmission. Larval catches of *A. varuna* were very considerably lower even than those of *A. fluviatilis*; it is



true that indications from adult catches were that its density was greatest in Yedadore but it was also by far the lowest in Byrapura. It did not seem probable that *A. fluviatilis* or *A. varuna* played more than a very small part in the transmission of malaria. The same may be said of *A. stephensi* which,

apparently, was most prevalent in Gargeshwari and least prevalent in Yedadore.

RACES OF *A. CULICIFACIES*.

Such variations in malaria as were found in these three villages might have been due to the existence of two or more races of *A. culicifacies* with differing efficiencies as malaria carriers, although it is rather a stretch of the imagination to expect such sharp divisions between races in so small an area. Careful examination of the ova of *A. culicifacies* caught in different parts of this area, and obtained from other parts of Mysore State, failed to reveal any dependable variations in egg pattern. The same was true of the ova of *A. fluviatilis* and *A. stephensi*. If races of these anophelines exist in Mysore, they cannot be separated on the basis of egg patterns. There seemed to be two distinct sizes of adult *A. culicifacies* but more careful study revealed intermediate sizes and no definite division was possible.

Sera for testing the source of mosquito blood meals did not become available before the close of this study.

A. CULICIFACIES AND HOUSING.

Of the eight anopheline catching stations in Gargeshwari, two were inhabited by humans only, three were combined human and animal habitations, and three were used for animals only—mainly for cattle, sheep, and goats. The corresponding stations in Yedadore and Byrapura were one human habitation, five combined human and animal dwellings, and two cattle-sheds. Table VII gives the average number of female *A. culicifacies* caught in a single station of each type in one hundred minutes, and the percentage of this catch was of the average total caught in three stations (one of each type) in the same time.

TABLE VII.

Average number of female A. culicifacies caught per single station in 100 minutes and percentage of the total caught.

Village.	HOUSE.		COMBINED HOUSE AND CATTLE-SHED.		CATTLE-SHED.		Total caught.
	Number.	Percentage of total in 100 minutes.	Number.	Percentage of total in 100 minutes.	Number.	Percentage of total caught.	
Gargeshwari	11	7.3 ± 1.4	78	51.7 ± 2.7	62	41.1 ± 2.7	151
Yedadore	17	10.0 ± 1.5	64	37.6 ± 2.5	89	52.4 ± 2.6	170
Byrapura	5	5.3 ± 1.6	34	36.2 ± 3.3	55	58.5 ± 3.4	94

The percentages of the average total catches of female *A. culicifacies* in 100 minutes made up of catches in a human habitation were significantly lower in all three places than the percentages made up of catches in the other two types of stations but did not differ from each other significantly. In

Gargeshwari the percentages caught in combined stations and in cattle-sheds did not differ from each other beyond the bounds set by chance, but the proportions caught in pure cattle-sheds in Yedadore and Byrapura were both significantly higher than the percentage caught in the combined houses and cattle-sheds in these areas. The percentage caught in Gargeshwari in 100 minutes in a combined house and cattle-shed was significantly higher than the corresponding percentages in either Yedadore or Byrapura, while the percentage caught in Gargeshwari in simple cattle-sheds was significantly lower than these percentages in the other two places. Table VIII gives the number and percentage of each type of edifice in the three villages under study.

TABLE VIII.

Number and percentage of edifice of three types and number of cattle per head of population.

Nature of edifice.	GARGESHWARI.		YEDADORE.		BYRAPURA.	
	Number.	Percentage of all edifices.	Number.	Percentage.	Number.	Percentage.
Human dwellings ..	195	59.3	74	41.8	58	43.0
Combined human and animal habitations ..	45	13.7	90	50.8	59	43.7
Cattle-sheds ..	89	27.0	13	7.3	18	13.3
All types of edifices	329	100.0	177	100.0	135	100.0
Population ..	1,486		658		563	
Number of cattle ..	1,568		481		425	
Number of cattle per head ..	1.06		0.73		0.75	

A study of the figures of Table VIII will show that Gargeshwari had a greater percentage than either of the other two places of houses used only for human habitation and cattle-sheds occupied only by animals and a lower percentage of combined human dwellings and cattle-sheds. Gargeshwari also had a greater number of cattle per head of population. Yedadore and Byrapura were approximately equal in their percentages of human dwellings, combined human and animal habitations, and number of cattle per head, but Yedadore had a smaller percentage of simple cattle-sheds.

MALARIA INFECTIONS IN ANOPHELES.

Dissections of female anophelines caught in daytime resting places were made during the month of June and, after an interval when no dissections

were made, were resumed on 16th September and continued till 12th December, 1935. The results of these dissections are given in Table IX.

TABLE IX.
Results of dissections of female anophelines.

Species.	Dates, 1935.	Number dissected.	OÖCYSTS IN STOMACH.		SPOROZOITES IN GLANDS.	
			Number.	Percentage.	Number.	Percentage.
<i>A. aconitus</i> ..	Sept. to Dec.	40	1	2.5	0	0.0
<i>A. culicifacies</i>	June	133	0	0.0	0	0.0
	Sept. to Dec.	1,058	45	4.3	8	0.8
<i>A. fluviatilis</i> ..	Sept. to Dec.	85	2	2.4	0	0.0
<i>A. stephensi</i> ..	June	1	0	0.0	0	0.0
	Sept. to Dec.	8	0	0.0	0	0.0
<i>A. varuna</i> ..	June	1	0	0.0	0	0.0
	Sept. to Dec.	6	0	0.0	0	0.0
All species ..	June	135	0	0.0	0	0.0
All species ..	16th Sept. to 12th Dec.	1,197	48	4.0	8	0.7

No malaria infections were found in any of the anophelines dissected in June 1935. Subsequent dissections began on 16th September and were carried on weekly until 12th December, during which period 1,197 females were examined. One stomach infection was found in *A. aconitus* and two in *A. fluviatilis*. This is the first report of infection in *A. aconitus* in Mysore State; in view of the findings and experience in other parts of the State it is not thought that this species is a natural carrier in this part of India. The great majority of stomach infections and the only gland infections were found in *A. culicifacies* and this species was undoubtedly responsible for practically all the malaria in the Gargeshwari area, sporozoites being found in the glands of 0.8 ± 0.2 per cent of the specimens dissected and some sign of malaria infection in 5.0 ± 0.5 per cent.

It was not possible to demonstrate any difference in *A. culicifacies* infection in the three villages of this area. From Gargeshwari 244 *A. culicifacies* were dissected, 439 from Yedadore and 375 from Byrapura. The percentages showing some sign of malaria infection in the three places were, respectively, 3.7 ± 0.8 , 6.4 ± 0.8 , and 4.3 ± 0.7 . Sporozoites were found in the glands of two specimens in Gargeshwari, six in Yedadore, and none in Byrapura.

The finding of either oöcysts or sporozoites was most common in the early part of the autumn dissections. Of 243 *A. culicifacies* dissected between 16th September and 3rd October some sign of infection was found in 24 specimens, 9.9 ± 1.3 per cent; between 4th October and 4th November the infection rate in 190 dissections was 3.2 ± 0.9 ; it was 3.7 ± 0.5 in 625 specimens

dissected between 5th November and 12th December. The numbers of mosquitoes found with sporozoites were so small in each of these periods that the percentages were not significantly greater than their probable errors.

Table X gives the results of dissections of *A. culicifacies* according to the daytime resting places in which the specimens were caught.

TABLE X.

Results of dissections of A. culicifacies according to place of capture.

Place caught.	Number dissected.	OÖCYSTS IN STOMACH.		SPOROZOITES IN GLANDS.		NUMBER OF MOSQUITOES INFECTED.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
House ..	3	2	..	0	..	2	..
Combined house and cattle-shed ..	480	15	3.1 \pm 0.5	2	0.4 \pm 0.2	17	3.5 \pm 0.6
Cattle-shed ..	575	28	4.9 \pm 0.6	6	1.0 \pm 0.3	34	5.9 \pm 0.7
TOTAL ..	1,058	45	4.3 \pm 0.4	8	0.8 \pm 0.2	53	5.0 \pm 0.5

Unfortunately only three *A. culicifacies* caught in human habitations were dissected; so no conclusions can be drawn from the fact that two of them were found to have oöcysts. There were no significant differences between the percentages of infection in the specimens caught in cattle-sheds and those captured in combined human and animal habitations. However, it is evident that malaria infections of *A. culicifacies* resting in cattle-sheds were not less than in those resting in combinations of human dwellings and cattle-sheds.

DISCUSSION.

Barber and Rice in two recent reports (1935*a* and *b*) have given valuable studies of anophelines in East Macedonia in relation to feeding and housing. *A. elutus*, the important natural malaria carrier of this area, when tested for source of its blood meals was found to be only moderately attracted to human beings, the percentages in the two reports being 38.8 and 42.0. (Total across Table 5 of 1935*a*, and across Table 5 of 1935*b*.) The percentages of *A. elutus* containing human blood when caught resting in houses were 61.3 and 59.6 but when caught resting in stables were only 7.5 and 8.8. To correspond with the feeding habits demonstrated by these blood tests, the catches of *A. elutus* in all houses and in stables were in the ratio of 1.0 to 2.8 (1935*b*, Table 1), and 1.0 to 3.4 (1935*a*, Table 7). In the case of *A. elutus*, therefore, its feeding habits could be judged, without a very serious error, from a knowledge of its daytime resting habits.

Barber and Rice (1935*b*) further state that sporozoite-infected specimens of *A. elutus* were just about as commonly found resting in stables as in houses (Table 6, 1935*b*). However, only 7.1 per cent of infected *A. elutus* caught

in stables contained human blood as against 47·5 per cent of those caught in houses (Table 6, 1935a). As far as the actual transmission of malaria is concerned the infected mosquitoes resting in stables were largely wasted due, first, to the low rate of preference of *A. elutus* for human blood and, second, to the risks inherent in a flight from stables to houses in which people would be available for biting. Conversely, the more separate stables there were, in an area in which the anopheline carrier had not a marked attraction to man, the safer the people would be from bites of infected mosquitoes.

No reports being available as to the blood preferences of *A. culicifacies* in South India, it may be unwise to proceed with this discussion but in view of the studies on *A. elutus* it would seem permissible to theorise to some extent. As the result of some years of experience in the study of malaria in various parts of Mysore State including the study of minor epidemics of malaria, one is led to predict that, like *A. elutus*, *A. culicifacies* in this part of India will be found to have no marked preference for human blood and that its efficiency as a transmitter of malaria is almost solely attributable to its density, the numbers of animals available, and the stabling of the animals. If this is proved to be true the most dangerous type of habitation would be found to be the combination of human dwelling and cattle-shed, so common in Mysore, in which people are readily accessible to infected *A. culicifacies* primarily attracted there by the presence of cattle, sheep, goats, etc. The area most free of malaria would be the one (given the necessary density in all areas) in which there were the greatest numbers of separate human dwellings and separate cattle-sheds and a minimum of combinations of houses accommodating both men and animals.

The remarks of the last paragraph would hold good, however, only if the feeding habits of the vector were the same throughout the year. It has been found in parts of Mysore (Sweet, 1933) that the period of most active transmission of malaria apparently corresponds to the season of the lowest number of *A. culicifacies*. It may be that the feeding habits of the female of this species are different in periods when she is pressed by the necessity of laying eggs from those when she is not so pressed, and that the explanation of transmission seasons in the tropics, where climatic conditions are always quite favourable, lies in varying degrees of attraction to man in different phases of the reproductive activity of the female. This seems to be the case in Holland with its autumn and winter transmission season (Swellengrebel *et al.*, 1936).

Gargeshwari had a higher percentage of separate houses and cattle-sheds and a larger number of cattle per head of population than either Yedadore or Byrapura but apparently the density of *A. culicifacies* was approximately the same as that in Yedadore as was also the percentage of infected anophelines. It is suggested that Gargeshwari is to a considerable extent protected from malaria by its housing conditions and that the malaria indices would have been even lower had there been no evacuation of the village for plague in 1934. As long as the density of *A. culicifacies* is not increased enough to overcome its housing advantage, Gargeshwari will probably remain comparatively free of malaria.

Housing conditions in Yedadore and Byrapura were not very different, except that the latter had more cattle-sheds, but in spite of this the latter village has a history suggesting that it is almost free from malaria, a fact not

true of Yedadore. Byrapura is situated on dry land with no irrigation near it and most of the anophelines present breed in the Kabbani River and one large nullah. It seems probable that the lower *A. culicifacies* catch in Byrapura in 1935 is a constant difference from Yedadore. It is suggested that the lack of malaria in this instance is due mainly to low anopheline density which is insufficient to support malaria without continuous replenishment from a chronic source such as was afforded by the bridge labourers' camp. It seems probable that Byrapura was provided with infected anophelines by this camp from towards the end of 1934 onwards and that malaria gradually increased. However, if the suppositions advanced are correct, it may be expected that Byrapura will continue to be malarious to a mild degree until climatic conditions reduce the *A. culicifacies* density below the requisite level for a sufficient length of time.

SUMMARY.

A study of malaria in three villages of Mysore State, lying within a circle with a radius of under one mile, was made during 1935. The parasite indices of children between 0 and 14 years of age were 6.9, 41.7 and 16.4 per cent with corresponding spleen indices of 21.0, 73.7 and 15.0 per cent. The catch of dangerous adult anophelines, the majority being *A. culicifacies*, in selected catching-stations was approximately equal in the first two villages but considerably lower on the whole in the third village.

Relative catches of infected and non-infected *A. culicifacies* in human dwellings, combined human and cattle houses, and in separate cattle-sheds suggested the possibility that *A. culicifacies* was not strongly attracted to human blood meals and that houses combined with cattle-sheds offered the greatest opportunities for malaria transmission by this species. The occurrence of malaria infection in *A. culicifacies* was about equal in all three villages and infected specimens were as common in separate cattle-sheds as in combined human and animal houses. It seems possible that one village was protected from severe malaria by the comparative absence of combined human and animal dwellings, and that another village usually enjoyed a low malarial incidence owing to a comparatively low density of *A. culicifacies*.

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AN IMPROVED FEATHER-DUSTER MOSQUITO TRAP.

BY

RAMKRISHNA N. GORE, L.M. & S.

[14th May, 1937.]

THE trap consists of a feather-duster of any dark shade, and an empty kerosene oil tin with the top removed (*vide* Fig. 1). A loose, detachable lid closely fitting at the top is provided, which can be fixed to the body of the tin by two latches. After removing the lid the duster is placed upside down in a diagonal position in the tin (*vide* Fig. 2). The tin is kept at night in a corner which has previously been observed to be a resting place of mosquitoes. One trap may be kept in each room. On the following morning, situations other than those where the trap is placed are disturbed at least twice between about 7 and 8-30 a.m. This is important since it helps in gathering more mosquitoes in the trap. By 10 a.m. the mosquitoes are at rest.

A piece of cloth is put over the mouth of the tin and by grasping the cloth and the feather-duster with one hand, the duster is removed with the other (*vide* Fig. 3). The lid is then placed over the tin and the cloth removed. The mosquitoes thus remain imprisoned in the tin.

The mosquitoes in the trap can conveniently be killed by placing the trap (which should be inverted) in direct sunshine. In the absence of a breeze all will be killed or moribund in about fifteen minutes.

The trap (still inverted) is brought into the house, the latches are undone, the tin raised, and the dead or moribund mosquitoes from the lid are transferred to a piece of paper and may be identified or destroyed by burning.

During the monsoon the mosquitoes may be killed by fumigation and heat. This is readily effected by burning a pinch (3 grains) of tobacco powder spread on a thin layer of cotton, rolled with a four-inch piece of wire and made into a sort of a small cigarette (*vide* Fig. 6). A hole three-fourths of an inch in diameter is made in the centre of one side of the trap. In a wooden plug one inch in length a hole half an inch deep is made. The end of the wire is inserted in the hole, and the 'cigarette', well lighted at the tip with a match or a live charcoal, is inserted through the hole in the inverted trap. After thirty minutes the mosquitoes are asphyxiated and can be removed on a piece of paper and

AN IMPROVED FEATHER-DUSTER MOSQUITO TRAP.

Fig. 1.

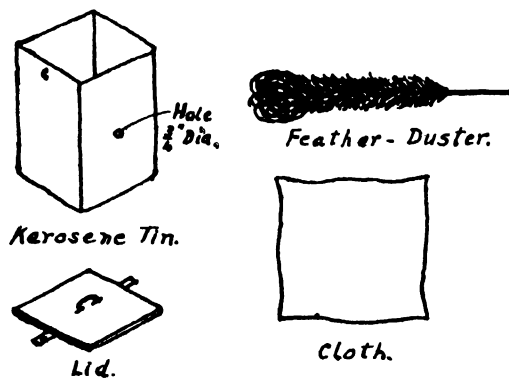
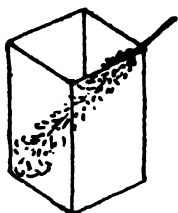
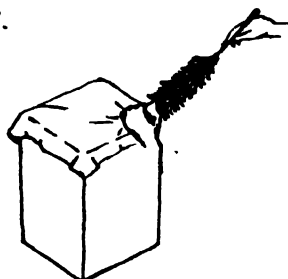


Fig. 2.



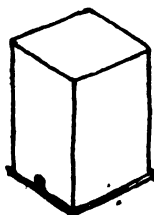
Feather-Duster in position.

Fig. 3.



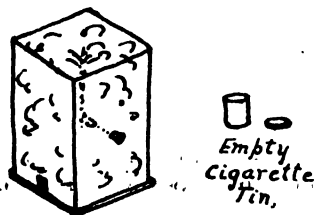
Removal of Feather-Duster.

Fig. 4.



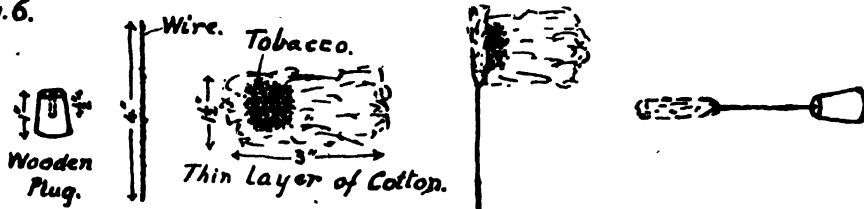
Killing by Sun Heat.

Fig. 5.



Killing by Fumigation and Heat.

Fig. 6.



put in an empty cigarette tin. Most of them die but 10 to 20 per cent remain in a moribund condition. Mosquitoes from other traps may also be transferred to this tin. The inverted lid containing a few pieces of live charcoal may be placed over the mouth of the small tin, and the heat thus generated is sufficient to kill the moribund mosquitoes.

ABSTRACT.

WINTER MALARIA INFECTION IN THE BENGAL DOOARS*.

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(MS. 16 pp. with one Sketch Map.)

[13th January, 1937.]

IN this paper the authors discuss the question of malaria infection in winter. They quote previous survey reports by Sur and Stewart (1926)† and by Iyengar (1931)‡ wherein the opinion is expressed that some mosquitoes probably continue to survive in artificially heated huts and are thus able to transmit malaria during the winter months.

The present investigation was commenced in April 1930 in the field laboratory of the Bengal Public Health Department at Sylee Tea Estate in the Jalpaiguri Dooars, three miles north of the Dam-Dim Station of Bengal Dooars Railway. The main part of the enquiry concerned the seasons in which infected anophelines were present in nature. For this purpose, a group of tea estates within a radius of six miles of the field laboratory was selected from which systematic collections of mosquitoes were made, once a week, from bungalows, Indian quarters and coolie huts. The seasonal variations of the different species of adult anophelines and the number of each species dissected for the years 1930 to 1935 are shown in Tables I and II. The relative prevalence of the mosquitoes is set out in Table III which shows that *A. minimus* was the most prevalent species, others in descending order being *A. vagus*, *A. maculatus*, *A. annularis*, and *A. philippinensis*.

* Copy of the original manuscript has been placed in the Library of the Malaria Survey of India, Kasauli. This is available on loan to workers who wish to consult the original. (Editor.)

† Sur, S. N., and Stewart, A. D. (1926). Report of the Malaria Survey of Jalpaiguri, Duars. Bengal Govt. Publication.

‡ Iyengar, M. O. T. (1931). The relative value of the oöcyst rate and sporozoite rate in anophelines. *Ind. J. Med. Res.*, 19, 2, pp. 525-539.

TABLE I.

Seasonal variations of the adult anophelines during the years 1930 to 1935 in the Dooars.

	<i>A. minimus</i> .	<i>A. varuna</i> .	<i>A. fluviatilis</i> .	<i>A. culicifacies</i> .	<i>A. jeyiporensis</i> .	<i>A. aconit</i> .	<i>A. maculatus</i> .	<i>A. splendens</i> .	<i>A. willmori</i> .	<i>A. tessellatus</i> .	<i>A. jamesi</i> .	<i>A. leucosphyrus</i> .	<i>A. kochi</i> .	<i>A. annularis</i> .	<i>A. philippinensis</i> .	<i>A. karwari</i> .	<i>A. maidi</i> .	<i>A. hyrcanus</i> .	<i>A. subpictus</i> .	<i>A. vagus</i> .	<i>A. atkeni</i> .
January ..	593	5	32	3	10	..	98	1	2	..	1	18	2	2	3	1	2	7	..
February ..	621	4	24	4	34	5	169	1	9	1	1	11	3	19	7	4	4	6	..
March ..	647	..	8	27	75	..	238	41	3	..	2	..	2	64	4	8	4	..	13	21	..
April ..	619	..	47	28	40	..	168	91	7	58	4	4	1	..	49	30	..
May ..	670	7	18	54	33	..	109	83	..	1	2	27	1	..	34	117	..
June ..	619	..	24	35	6	..	98	57	10	45	1	..	1	4	68	396	..
July ..	1,209	3	14	31	4	..	201	16	1	..	15	1	..	19	1	4	..	1	19	429	3
August ..	988	1	..	4	9	..	75	1	1	2	4	1	218	1
September	1,032	1	5	..	60	1	1	..	12	2	5	..	2	527
October ..	824	1	..	0	7	..	122	4	1	..	3	1	..	58	101	22	253	..
November	1,140	0	6	1	8	..	100	6	20	34	17	13	4	2	1	64	..
December	1,082	7	15	1	5	2	115	3	4	23	10	3	4	0	7	18	..
Total ..	10,044	28	188	189	236	7	1,553	304	5	1	75	4	4	370	147	84	26	12	199	2,086	4

TABLE II.
The various species of anophelines which were dissected during the years 1930 to 1935.

	<i>A. minimus.</i>	<i>A. varuna.</i>	<i>A. fluvialis.</i>	<i>A. culicifacies.</i>	<i>A. jayporensis.</i>	<i>A. maculatus.</i>	<i>A. jameesi.</i>	<i>A. annularis.</i>	<i>A. philippinensis.</i>	<i>A. karwari.</i>	<i>A. mayidi.</i>	<i>A. hyrcanus.</i>	<i>A. subpictus.</i>	<i>A. vagus.</i>	<i>A. splendens.</i>	Total.
January ..	538	3	23	1	9	82	3	12	2	2	3	1	1	680
February ..	524	3	18	2	23	147	1	11	2	11	3	1	3	3	1	753
March ..	551	..	7	15	53	149	1	46	4	2	1	..	1	7	29	866
April ..	492	..	32	12	27	172	3	54	3	1	60	856
May ..	607	4	15	44	27	73	32	12	9	31	77	931
June ..	478	..	18	16	10	74	9	42	1	..	11	33	50	742
July ..	815	..	6	27	1	116	9	15	1	2	20	11	1,023
August ..	912	1	..	4	9	84	2	1	..	3	24	..	1,040
September ..	902	1	..	50	1	11	1	1	1	31	..	999
October ..	720	5	100	3	37	59	6	6	3	939
November ..	787	..	2	..	6	77	17	26	13	23	3	3	4	961
December ..	1,059	3	12	2	10	66	3	18	7	1	4	2	1,187
TOTAL ..	8,385	14	133	124	180	1,190	84	285	101	52	16	2	15	158	238	10,977

TABLE III.
Percentage of the various species of anophelines caught during the period 1930 to 1935.

	1930.		1931.		1932.		1933.		1934.		1935.		Total.
<i>A. minimus</i>	404	52.5	1,101	46.4	1,883	61.2	2,456	77.6	1,614	63.1	2,586	70.6	10,044
<i>A. vagus</i>	118	15.3	587	24.7	308	10.1	119	3.7	334	13.0	620	16.9	2,086
<i>A. maculatus</i>	87	11.3	336	14.1	397	13.0	269	8.4	231	9.0	233	6.3	1,553
<i>A. annularis</i>	24	3.1	136	5.6	111	3.6	38	1.2	42	1.6	19	0.51	370
<i>A. splendens</i>	103	4.3	81	2.6	61	1.8	40	1.5	19	0.51	304
<i>A. jayporiensis</i>	8	1.0	13	0.5	21	0.6	28	0.88	87	3.4	79	2.1	236
<i>A. subpictus</i>	52	6.7	4	0.16	38	1.2	34	1.06	48	1.8	23	0.62	199
<i>A. culicifacies</i>	2	0.2	2	0.08	46	1.5	14	0.44	83	3.2	42	1.1	189
<i>A. fluviatilis</i>	63	8.0	17	0.7	27	0.9	60	1.8	16	0.62	5	0.13	188
<i>A. philippinensis</i>	11	0.46	92	2.6	18	0.56	16	0.62	20	0.54	147
<i>A. karwari</i>	1	0.1	14	0.5	13	0.4	30	0.94	18	0.70	8	0.21	84
<i>A. jamei</i>	5	0.7	34	1.4	16	0.5	10	0.31	7	0.27	3	0.08	75
<i>A. varuna</i>	8	0.3	4	0.13	9	0.28	7	0.27	28
<i>A. majidi</i>	4	0.16	7	0.2	9	0.28	5	0.19	1	0.02	26
<i>A. hyrcanus</i>	5	0.7	2	0.06	2	0.06	1	0.03	2	0.05	12
<i>A. aconitus</i>	2	0.06	5	0.19	7
<i>A. willmori</i>	3	0.09	2	0.07	5
<i>A. leucosphyrus</i>	1	0.04	2	0.06	1	0.03	4
<i>A. kochi</i>	3	0.09	1	0.03	4
<i>A. aulkeni</i>	2	0.08	1	0.03	1	0.02	4
<i>A. tessellatus</i>	1	0.03	1
TOTAL	769	..	2,373	..	3,041	..	3,165	..	2,557	..	3,661	..	15,566

TABLE IV.
Statement showing dissection of A. minimus.

Statement showing distribution of influenza																						
	1930.			1931.			1932.			1933.			1934.			1935.			Combined rate of infection.			
	Number diseased.	Gland infection.	Combined rate of infection.	Number diseased.	Gut infection.	Gland infection.	Number diseased.	Gut infection.	Gland infection.	Number diseased.	Gut infection.	Gland infection.	Number diseased.	Gut infection.	Gland infection.	Number diseased.	Gut infection.	Gland infection.				
January	145	..	8	5.5	166	1	1	1.20	91	..	1	1.09	136	1	1	1.47
February	113	156	1	1	1.28	121	2	5	5.78	134	1	3	2.98
March	114	5	2	6.14	215	4	2	2.79	81	..	6	7.40	141	1	4	3.54
April	81	1	5	7.40	234	6	12	7.69	66	2	4	9.09	111	1	2	2.70
May	7	241	..	12	4.97	204	..	11	5.39	61	1	5	9.83	94	..	1	1.06
June	12	116	1	5	5.17	130	3	10	10.0	79	2	9	13.92	141	..	3	2.12
July	36	..	2.77	77	..	3	100	..	5	5.00	275	..	10	3.63	127	1	6	5.51	200	..	10	5.00
August	69	3	4.34	165	..	17	123	..	9	7.31	161	..	1	0.62	152	..	9	5.92	242	1	14	6.19
September	79	12	15.18	184	..	11	284	6	25	10.91	111	1	1	1.80	79	..	7	8.86	165	4	13	10.30
October	95	3	3.15	78	..	7	91	1	7	8.78	111	3	5	7.20	139	1	21	15.80	206	..	15	7.28
November	49	3	6.12	233	..	14	66	1	3	6.06	148	..	8	5.40	131	2	3	3.81	160	5	13	11.25
December	32	144	..	14	235	3	13	6.80	170	2	8	5.88	207	..	12	5.79	271	11	19	11.07
Total	360	22	6.11	900	..	66	1,709	18	94	6.55	2,081	21	70	4.37	1,334	11	88	7.42	2,001	25	98	6.14

The results shown in Tables II and IV indicate that infected mosquitoes occurred during every month of the year and that *A. minimus* was the chief malarial vector. Out of 8,385 specimens of this species dissected during the six years under review, oöcysts and sporozoites were found in 513 (*vide* Table IV). The infection rates in different months for the years 1930 to 1935 are shown in Table IV. Other species found infected were *A. culicifacies* (1 specimen), *A. annularis* (1 specimen), and *A. philippinensis* (2 specimens).

In addition, a spleen and parasite survey among the infants born during the period November 1935 to February 1936 was made in seven tea estates. A total of 97 infants was examined and the results of this investigation are shown in Table V.

TABLE V.

	Number of infants examined.	Infants with enlarged spleen.	Infants showing parasites in peripheral blood.
1. Sylce Tea Estate ..	16	4	1
2. Needeem ..	12	1	1
3. Baint-Bari ..	16	2	2
4. Dalimkota ..	6	1	0
5. Meenglas ..	18	5	2
6. Ranichera ..	14	4	4
7. Bulla-Bari ..	15	3	1
Total ..	97	20	11
PERCENTAGE	20.6	11.3

Out of a total of eleven infants showing parasites in their blood, three were born in the first week of November and one in the last week of November. Of the remaining, two were born on the 1st and 2nd December respectively, while five were born between the last week of December and the end of February. Even allowing for the small number of infants, definite evidence is presented to show that malaria transmission occurs in the winter months.

In the discussion, reference is made to the investigations by Manson and Ramsay (1932)* who had found no infected mosquitoes during the months of January, February, and March in tea estates in Sibsagar District of Assam. The findings in the field laboratory at Jalpaiguri, Dooars, on the other hand, are contrary to these results.

* Manson, D., and Ramsay, G. C. (1932). Some findings in a malaria survey carried out on a group of tea estates in the Sibsagar District of Assam from August 1, 1930 to July 31, 1931. *Rec. Mal. Surv. Ind.*, 3, 1, pp. 143-160.

Records of meteorological data such as temperature and humidity are also presented. It is claimed that these show that in the Bengal Dooars these factors do not remain continuously unfavourable and are such that they can allow the development of parasites in the mosquitoes throughout the year.

M. K. A.

ANTI-GAMETOCYTE TREATMENT COMBINED WITH ANTI-LARVAL MALARIA CONTROL.

Part II.

BY

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[17th June, 1937.]

1. INTRODUCTION.

IN the first part of this paper (Senior White and Adhikari, 1934) the authors showed that no permanent improvement in either child or adult malaria incidence is achieved by a single blanket-treatment attack on gametocytes in children, prior to the start of a transmission season throughout which anti-larval measures are in force, compared with the results achieved in former years by anti-larval measures alone.

The obvious corollary to such an experiment is to repeat it in a subsequent season, exhibiting the anti-gametocyte treatment more frequently, and giving it not only to the children, but also to the adults who as Hackett (1937) states 'become carriers off and on, and the havoc they work is out of all proportion to their numbers'. But under industrial conditions, without a special grant for the necessary additional staff, it is impossible to arrange for such an experiment. When the elaborate organisation described by the present authors for their first experiment with a single course of treatment is considered, it is manifestly impossible to envisage the continuation of such an experiment over several months. As is the case everywhere in Asia, women present great difficulties in treatment, and since under railway conditions there is no period of the twenty-four hours when all the inhabitants of a station are available at the same time, great difficulties are encountered in mustering them for

treatment. Furthermore, local villagers are potential carriers, on whom there can be no compulsion, but at most some official persuasion, so that it could not be anticipated that effective blanket-treatment could be maintained over a period of several months.

Fortunately, however, some years after our first experiment referred to above, an isolated camp in the same district where compulsion could be applied and which was far removed from any village, became available for experimental purposes. This camp was established in connection with further work on Saranda tunnel, on the main line from Calcutta to Bombay, situated not more than 28 miles in a direct line from Dangoaposi, the scene of our first experiment.

The early history of the construction of Saranda tunnel has been briefly described in a paper by Senior White (1928) in which (Plate III) a sketch survey plan of the site is given. With the completion of the new down line tunnel in 1929 the station was closed, as the passing loop-line was no longer required, and the area reverted to its primeval isolation. The only inhabitants were the watchmen who were on duty at the four tunnel entrances and who lived in their villages at some distance from the line.

In 1934, however, the strata in the new tunnel commenced to give trouble. There was a tendency for slips to occur and for portions of the rock to fall on the track. The tunnel had to be patrolled after every train had passed. On the advice of the Geological Survey of India it was decided to brick-line portions of the tunnel. This, of course, involved some further excavation of the original rock to make room for the brick work. This was carried out between June and October 1935.

The camp established in connection with this work was only a small one, some 160 strong on an average, and was located east of the tunnels on the site of the former station. Anti-larval measures which were carried out within a half-mile radius (in 1926-1929 only a quarter-mile radius was tried) were brought into force well before the camp was occupied. In addition, it was planned to give everyone in camp a weekly dose of plasmochin as an anti-gametocyte measure, to treat all those who showed fever with a 5-day course of atabrin, and to control all results by blood examinations. However, the Sub-Assistant Surgeon employed was so idle and so incompetent in keeping records that his results were entirely useless. Although a certain amount of treatment was given it may be taken that such results as were achieved were brought about almost entirely by the anti-larval measures with which this Sub-Assistant Surgeon had nothing to do, but which were in charge of an Inspector of the Malaria Section of this Department. The health of the camp was good but as a drug prophylaxis experiment nothing was achieved.

A second opportunity, fortunately, occurred in 1936 owing to further trouble with the strata involving the brick-lining of almost the whole remaining length of the down tunnel. The Sub-Assistant Surgeon posted on this occasion was specially selected by the Chief Medical Officer, and instructed in advance by us in his record keeping duties. The same Malaria Inspector was placed in charge of the anti-larval measures, which were brought into force two months before the camp (on the same site as in the previous year) was occupied.

2. METHOD OF RECORDING RESULTS.

The following records were maintained :—

- (1) Weekly adult anopheline catches at two points in the protected (camp) area, and at two points to the west of the tunnels outside the controlled area, 20 minutes each week being spent on each catching station.
- (2) Monthly dissections of wild anophelines caught in a house occupied by a track-maintenance gang, and in a village about a quarter of a mile away from it, both beyond the protected zone, to indicate the transmission season and the severity of the local infection rates.
- (3) Examination of the blood of every arrival in the camp, within 24 hours of taking up residence.
- (4) Examination of the blood of everyone who reported at the hospital tent for 'fever'.
- (5) Examination of the blood of everyone leaving the camp on completion of his period of service therein. All arrivals, whether showing malaria parasites or not (this was not known to the Sub-Assistant Surgeon at the time, as the slides had to be sent to Calcutta for examination), were treated with a 5-day course of atebirin (0.3 gm. daily), followed, after a 5-day interval, by 0.02 gm. plasmochin simplex on one day. Thereafter, everyone was given 0.02 gm. plasmochin simplex on one day each week.
- (6) This 'prophylactic' treatment was entered in the prophylactic treatment-register, man by man, each week. From this register it has been computed that 73.2 per cent were successfully treated every week of their stay. The remainder missed one or more weeks treatment owing to their temporary absence from the camp. Thus if untreated persons became gametocyte carriers they did not serve as sources of infection, since they were absent from the camp. When a person reported sick with fever, a blood film was at once taken and despatched to Calcutta, and meanwhile he was placed on a 7-day course of 0.3 gm. atebirin per diem, followed, after a 5-day interval, by a 5-day course of 0.02 gm. plasmochin daily.
- (7) Such persons' names were transferred during this course from the 'prophylaxis' to the 'treatment' register, only reverting to the former on completion of the curative course. From the register of the latter it has been found that 91.5 per cent of those showing fever completed curative courses.

In the case of a second, or subsequent, attack of fever additional blood films were taken, but no further 'curative course' was exhibited. Such persons remained on prophylactic plasmochin.

For second and later relapses atebirin was not used, but quinine in doses of 15 grains daily was exhibited, the patient continuing to receive his weekly plasmochin as if further relapses had not supervened. Probably the quinine mixture was only taken by the class of persons involved during and immediately after the attack, and was without any curative value.

The vast majority of the inhabitants of the camp were men. Among the skilled labourers many races were represented. The unskilled labourers were

local aborigines (Hos, Mundas and Oraons). As the women of these tribes refused any kind of medication, they were not permitted to sleep in the camp. In consequence there were very few children in the camp, such as there were being in the main those of the railway subordinate-supervising and electrical staff of less than a dozen persons.

3. RESULTS.

The results obtained are given below in tabular forms. Perusal of these tables and of the explanatory notes will make the findings clear.

(1) 'TURN-OVER' OF LABOURERS IN THE CAMP.

TABLE I.

Length of residence in camp (Contractor's Labour only).

Period in weeks.	Number.	Percentage.
0-3 ..	135*	20.4
4-6 ..	92	13.9
7-9 ..	76	11.5
10-12 ..	52	7.8
13-15 ..	80	12.1
16-18 ..	48	7.3
19-21 ..	24	3.6
22-24 ..	31	4.7
25-27 ..	28	4.2
28-30 ..	41	6.2
31-33 ..	34	5.1
34-36 ..	21	3.2
Total .	662	-

*Includes one man who died within 24 hours of arrival, without blood being taken.

Although the average labour strength employed on these works was only 308, the amount of labour 'turn-over' to maintain this strength was considerable. It was found that one-fifth of the total force was changed over within the first month, and that within three months over one half of the force abandoned the work probably because they had earned sufficient money to meet their immediate needs. Of the remainder a high proportion apparently required four months to achieve their financial object.

The result of this tendency on the part of the labourers to abandon the work was most unfortunate from our point of view. Many workers left the camp without visiting the doctor for a final blood film and the records of their state of health on leaving were therefore incomplete.

The extent of the constant 'turn-over' of labour which may take place in connection with engineering projects is indicated by the figures in Table I which provides a lesson of economic as well as of medical value. Every new-comer is a potential source of some infective disease and if any such disease

is to be the subject of prophylactic measures such as anti-cholera injections, anti-hookworm campaigns, or, as in the present instance, of anti-malaria treatment, it is clear that there must be a great deal of wastage of drugs, vaccines, etc., on those who leave the work shortly after arrival.

(2) INDIVIDUALS INFECTED WHEN LEAVING THE CAMP.

TABLE II.

Showing the percentage of persons whose blood showed no malaria parasites on arrival but who were infected when leaving the camp.

Length of residence in weeks.	Number in each category.	Number infected when leaving camp.	Percentage
0-3 ..	125	3	2.4
4-6 ..	81	7	8.6
7-9 .	59	4	6.8
10-12 ..	39	4	10.2
13-15 ..	61	10	16.4
16-18 ..	40	2	5.0
19-21 ..	21	4	19.0
22-24 ..	32	0	0.0
25-27 ..	24	6	25.0
28-30 ..	33	7	21.2
31-33 ..	28	2	7.1
34-36 ..	17	2	11.8
Total .	560	51	9.1

Table II does not give an accurate picture of the extent of locally acquired malaria infection. A certain number of those whose blood showed no parasites on arrival were probably in a latent stage of infection without parasites in the peripheral blood. But against any deduction on this score must be placed persons who showed active infection (including relapse cases) during their stay, and who were treated successfully so as to be actually negative on departure. There were 84 such persons details of whom are given in Table IV*.

* Conditions on the works were extremely conducive to the bringing out of any latent infection. The work in the tunnel was extremely arduous. There was a great deal of water percolation through the roof, soaking everyone to the skin. In the earlier months the tunnel was, by comparison with the conditions outside, distinctly chilly. In December and January, on the other hand, external conditions locally were definitely cold out of the sun. Day and night shifts were worked.

(3) MALARIAL INFECTIONS TREATED IN THE CAMP.

TABLE III.

Parasite findings among persons who showed parasites on arrival, when leaving camp.

				Total arrivals showing <i>P. vivax</i>	57	} = 101
				" " " <i>P. malariae</i>	3	
				" " " <i>P. falciparum</i>	41	
Persons with <i>P. vivax</i> infections who were negative on leaving the camp	..	21						} = 57
" " " " " " showing <i>P. vivax</i> on leaving the camp	..	0						
" " " " " " " <i>P. falciparum</i> on leaving the camp	..	2						
" " " " " " absconded	34						
Persons with <i>P. malariae</i> infections who were negative on leaving the camp	..	1						} = 3
" " " " " " absconded	2						
Persons with <i>P. falciparum</i> infections who were negative on leaving the camp	..	13						} = 41
" " " " " " showing <i>P. falciparum</i> on leaving the camp	..	5						
" " " " " " " <i>P. vivax</i> on leaving the camp	..	0						
" " " " " " absconded	23						

Owing to large numbers who absconded without a final blood film being taken, much of the value of these figures is lost. All persons found infected on arrival were immediately placed on curative treatment, which was apparently successful in eradicating (with the subsequent prophylactic plasmochin) 91 per cent of the *vivax* and 72 per cent of the *falciparum* infections brought into the camp.

(4) MALARIAL INFECTIONS ACQUIRED IN THE CAMP.

TABLE IV.

Statement showing the probable number of malarial infections acquired in the camp.

Month.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Totals.
<i>P. vivax</i>	3	1	4	3	4	1	2	9	27
<i>P. falciparum</i>	8	6	5	7	3	5	11	12	57
Total	11	7	9	10	7	6	13	21	84

Table IV is composed of (a) persons whose blood was negative on arrival, and (b) persons who showed a change of parasite during their stay in the camp from the species they harboured on arrival. Both classes are subject to errors due to latent infections on arrival. The January figures are raised by persons who had never reported sick whilst in the camp, but whose blood on final examination was positive. There were 14 such infections (*P. vivax* 4, *P. falciparum* 10) leaving only seven active infections for January. December is thus seen to be the month of maximum infection, as revealed by the number of malaria cases. This does not agree with the mosquito infection rates shown in Table VII.

(5) TREATMENT OF FEVER CASES.

A total of 147 persons reported at the hospital tent with fever due either to locally contracted infections, or to infections discovered by the initial blood examination*. Details of these cases are given in Table V.

TABLE V.

Showing cases which received curative courses of treatment.

Blood examination.	Number.	Percentage.
<i>P. vivax</i>	61	41.5
<i>P. malariae</i>	2	1.3
<i>P. falciparum</i>	66	44.9
No parasite found	18	12.3
Total number examined ..	147	..

All underwent the curative course, 91.5 per cent completing it. Of these only 22 (15 per cent) relapsed, of whom the details are given in Table VI.

TABLE VI.

Relapses after treatment.

	Interval after initial detection of infection at which relapses occurred, in days.							Total number of relapses observed†.
	≤21	≤42	≤63	≤84	≤105	≤126	>126	
Days after treatment on which relapses of <i>P. vivax</i> were observed.	14 14 15	29	46 56 ..	75	90 97 ..	116	150	
Total relapses at each interval.	3	1	2	1	2	1	1	11
Days after treatment on which relapses of <i>P. falciparum</i> were observed.	28 35 39	45 46 52 53 58 61 65 ..	69 76 77	95 97 104 104	106 107 109 114 118	144 146 150 167 169 184 196 210 220	
Total relapses of <i>P. falciparum</i> at each interval.	0	3	7	3	4	5	9	31

* Persons found infected on arrival not only got the original blanket-treatment, but a further curative course.

† The total figures are for the number of relapses, not of individuals relapsing.

In the *vivax* group seven persons relapsed once, and two relapsed twice. In the *falciparum* group sixteen relapsed once, four relapsed twice, one relapsed three times, and one relapsed four times. In the last case relapses occurred at 76, 97, 109, and 150 days from the initial finding of the parasite. This man was still positive when paid off after 196 days.

At first glance, Table VI is unusual, in as much as there were far fewer *vivax* relapses than *falciparum* relapses. But, it has to be borne in mind that whilst plasmodium is active against all stages of *vivax*, it is quite inert as regards the asexual forms of *falciparum* (Schulemann *et al.*, 1932). Thus a *vivax* patient had the asexual stage of his parasites under continual, if very mild, medication, whilst a *falciparum* patient did not. Hence the latter parasite showed three times as high a relapse rate as the former, which is quite contrary to usual experience. But it is likely that many of the so-called *falciparum* 'relapses' were actually re-infections. Perhaps most of those shown as occurring at over 105 days (15 weeks) from the initial detection of parasites are actually re-infections, for after the fourteenth week most cases of *P. falciparum* cease to relapse (James, Nichol and Shute, 1936).

(6) ANOPHELINE INFECTION RATES.

TABLE VII.

A. funestus-group infection rates.

Month.	Number dissected.	Gut infected.	Glands infected.
1936-1937.			
May ..	0	0	0
June ..	0	0	0
July ..	1	0	0
August ..	12	0	0
September ..	16	1	0
October ..	120	6	2
November ..	129	5	0
December ..	127	1	1
January ..	73	1	0
Total ..	478	14	3

All three species of the group (*A. fluviatilis*, *A. varuna* and *A. minimus*) were found infected. In addition 203 *A. culicifacies* were dissected, but none was found to be infected. After November, the latter species entirely disappeared from the catches. Transmission of malaria did not apparently commence before September, though as the carrier species were present in small

numbers throughout, as is shown in Table VIII, it is not impossible that a low degree of transmission occurred as soon as the rainy season commenced. In 1936 the rains started in May which is exceptionally early in the year for this locality.

(7) ADULT ANOPHELINE CATCHES.

TABLE VIII.

*Comparative adult check catches, controlled area and uncontrolled area.
(Two stations in each throughout.)*

Month.	Number of catches in each area.	CONTROLLED AREA.				UNCONTROLLED AREA.			
		<i>culicifacies.</i>		<i>funestus</i> group.		<i>culicifacies.</i>		<i>funestus</i> group.	
		♂	♀	♂	♀	♂	♀	♂	♀
1936-37.									
March ..	1	0	1	..		0	2	0	1
April ..	4	1	0	0	1	0	1	..	
May ..	5		2	0	0	1
June ..	4		0	1	0	3
July ..	4		1	2	0	5
August ..	5		6	10	0	3
September ..	4	1	0	..		0	5	0	6
October] ..	5		0	1	0	30
November ..	4	..		0	2	0	1	0	39
December ..	1	..		0	1	..		0	9
Total ..	37	2	1	0	4	9	23	0	97

A general strike, which commenced in the first week of December, so disorganised train services that the sending of weekly catches to Calcutta for identification had to be discontinued, and the records are therefore incomplete for the remainder of the period.

The anti-larval measures are seen to be very effective causing a 24 : 1 reduction in *funestus* incidence in the protected as compared with the unprotected area. None-the-less 84 cases of infection apparently occurred in the nine months during which the camp was occupied (Table IV), though the infection rates of the carrier species were much lower than was found for the same group of species in the Jeypore Hills (Senior White, 1937).

(8) GAMETOCYTE INCIDENCE.

A study of Table IX shows (a) that the gametocyte rate was the same on departure as on arrival, and (b) that unmedicated newly infected adults have the same gametocyte rate as newly infected adults who have been previously on prophylactic plasmochin.

TABLE IX.

Place.		GAMETOCYTE INCIDENCE.				
		Number.	Species.			Gameto- cyte rate.
			<i>P. vivax.</i>	<i>P. malariae.</i>	<i>P. falciparum.</i>	
Saranda camp.	Slides found positive on arrival.	101	6	1	1	7.0
	Locally acquired infections.	84	1	0	1	2.4
	Slides found positive at close of work.	38	2	0	1	7.1
Dangoaposi	Employees reporting sick with positive slides (1928-37).	428	2	4	5	2.6

4. SUMMARY OF RESULTS.

Owing to the absence of any comparative figures (even the badly kept records of 1935 cannot be used as the camp was then only occupied from June to October, and thus escaped the worst of the infection season) no deductions can be drawn from these results regarding the ancillary efficiency of the anti-gametocyte measures over anti-larval measures alone. All we can say is that with both these measures in force the work proceeded from start to finish without any delay whatever due to malarial sickness, whereas we know from the history of earlier work in this place (Senior White, 1928) that uncontrolled malaria will work havoc with engineering operations.

The cost of the drugs used amounted to Rs. 1,413, or Rs. 2-2-0 per person employed on the works, or Rs. 4-9-0 per head of average effective strength, whilst the corresponding figures for the anti-larval works (in this case including materials, local supervision and labour) were Rs. 6,428, Rs. 9-12, and Rs. 20-14, respectively. This latter figure is high, but the protection could have covered a much larger force without additional cost. It happened, owing to the size of the camp, that the Sub-Assistant Surgeon in general medical charge could do drug prophylaxis in addition to his other duties. On a larger work, with more camps under one Sub-Assistant, this would not be the case, and the cost of drug administration would rise accordingly. Again, in this instance, there was no village within the range of mosquito flight from the camp. Where there is such, with its constant supply of parasites, it would appear that supplementary anti-gametocyte measures are not feasible.

5. CONCLUSION.

There appears to be no additional benefit from anti-gametocyte measures over anti-larval measures alone, commensurate with the extra 22 per cent *per capita* cost of protection that the former involves.

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QUININE SUPPLIES IN INDIA.

BY

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[15th July, 1937.]

I. INTRODUCTION.

THE discovery and commercialisation of the cinchona plant make one of the most interesting stories associated with the development of modern medicine. For nearly two centuries after it became known that cinchona bark contained an active and valuable febrifuge, unbridled exploitation of the cinchona forests in South America went on until these enormous natural stores of the drug were in danger of becoming exhausted.

In order to combat the danger of scarcity of the bark, a Dutch expedition in 1852 and an English one in 1860 went to South America to obtain supplies of cinchona seeds and plants with the object of opening up cinchona plantations in Java and India. The British expedition succeeded in delivering in India over 450 live plants and 100,000 seeds of *C. succirubra* and the Dutch expedition achieved equally successful results. Within a few years by mutual exchange both countries possessed a number of different varieties of the cinchona plant and successful transplantation was followed by a period of experimentation and research in respect of the best methods for propagation of those species giving the higher percentages of quinine. Thanks to the determination of an Englishman and his Indian servant, a collection of seeds of *C. ledgeriana*, which gives the highest percentage of quinine, was made available to both the Indian and Dutch Governments.

Until this variety was successfully transplanted, the cultivation of cinchona both in India and in Java had shown little prospect of commercial success and it had been impossible to induce private companies to take part. In view, however, of the ready demand and the higher prices obtainable for ledgeriana bark, the position completely changed and private plantations were successfully developed not only in Java but also in Ceylon and South India. By 1880, instead of a shortage, the supply of cinchona bark was considerably greater than the demand, and in the following years the price continued to fall until in 1895 it had reached a level below that of the cost of production. As a result, both in Ceylon and South India the planters found themselves compelled to give up cinchona cultivation in favour of tea and the Dutch East Indies was left

practically the sole producing country. For many years past Java has produced approximately 97 per cent of the total world supplies, India being responsible for about 2·5 per cent and other countries for the minute proportion of 0·5 per cent. That in brief is the history of cinchona cultivation since the discovery of the plant in South America.

II. THE QUININE QUESTION FROM A WORLD ECONOMICS POINT OF VIEW.

It is obvious that production of quinine has not only a humanitarian aspect but that it is also a world economics problem. From the humanitarian point of view it is desirable that every sufferer from malaria, even the poorest, should be able to obtain sufficient quinine for his needs. Available statistics make it possible to give an approximate estimate of the numbers of persons in the world infected with malaria. In 1925, the Malaria Commission of the Health Committee of the League of Nations estimated that no less than 26,000 tons or 58,240,000 lbs. of quinine would be required annually in order to provide 40 grains to every malaria case in the world. Present world consumption, according to recent reliable information, reaches a figure of only 600 tons or 1,344,000 lbs. although this total could be increased to 1,400 tons or 3,136,000 lbs. if present restriction of production was removed by the Java authorities. It must be remembered that millions of malaria patients are so poor that they cannot possibly afford to buy quinine even though the price was reduced to the cost of production. From the world economics point of view, the problem is two-fold; the supply of sufficient quinine at a price low enough to meet the needs of poverty-stricken malaria sufferers and, at the same time, the maintenance of quinine production on an economically sound basis.

Every endeavour must be made to place the present large stocks of quinine at the disposal of malaria sufferers in an inexpensive and convenient way. This is a public health problem of the first importance. On the other hand, until those interested in the control of malaria take into account the conditions that make quinine production economically possible, discussions on lower prices and larger production may well be of little avail. As has already been indicated, at the end of last century the principal cause of continued reductions in the price of cinchona bark was 'over-production', the supplies of bark greatly exceeding the demand, because of the lack of organised co-operation amongst the planter producers. On the other hand, because quinine manufacturers had already reached a sufficient degree of co-operation, they became the real masters of the cinchona market and had the power to fix prices as they chose. During 1910-1912, the price of bark again fell to such an extent and the Java producers were in such a critical position that they finally agreed to combine, and in 1913 their organisation came to an agreement with the quinine manufacturers which provided a basis of co-operation. This co-operation has been maintained by fresh agreements, the control and execution of which is entrusted to the Kina Bureau in Amsterdam.

The Dutch authorities maintain that the Cinchona Agreement has never been used to force prices of quinine to an unreasonable level and quote, as an instance, the War Agreement made with the Allies under which the latter 'secured practically the whole year's production of Java quinine on reasonable terms', the price received being far below the market price at the time. The Government of the Netherlands East Indies, it is stated, 'has always adopted the standpoint that only a reasonable profitable industry can be the sound basis

for a normal and constant production'. Proof that that Government desires to safeguard against any possible abuse is given, it is said, in Article 117 of the Cinchona Agreement, which was included because the Netherlands East Indies Government made it a condition of their signing the Agreement. This article reads: 'The Government of the Netherlands East Indies reserve the right to surrender membership of the Association at a year's notice if the Government are of opinion that the Cinchona Agreement forms an impediment for the supply of quinine to the malarial districts'. It must be remembered that the Netherlands East Indies Government are themselves owners of large cinchona plantations which produce about 10 per cent of the entire supplies of Java bark.

At different times, the Kina Bureau has also made contracts for the supply of cheap quinine with Albania, Bulgaria, Greece, Russia and Italy and has asked for the co-operation of the Protestant and Roman Catholic Missions to bring cheap quinine to malaria sufferers in China and the Belgian Congo.

It is obvious, in the interest of the producers themselves, that consumption of quinine should be increased, such as can be induced by a systematic supply of cheap quinine to malaria infected regions. Dutch interests, it is said, 'cannot lie in a "trust" for forcing up prices but rather in the sale of as much quinine as possible at prices at which profitable cultivation can be permanently maintained'.

The final argument produced by the Dutch authorities is that :—

'without the Cinchona Agreement, quinine would once more become a speculative article in the hands of middlemen, who would be much more interested in the supply of quinine to markets where the largest profits could be made rather than in supplying cheap quinine to impoverished sufferers from malaria. And the various Governments interested in quininisation of malarial regions, would not then have the assurance that they could regularly obtain adequate supplies of cheap quinine, a certainty which the Cinchona Agreement now furnishes'.

III. THE POLICY OF THE GOVERNMENT OF INDIA.

After the introduction of cinchona in 1861, for many years the object was to manufacture from Indian grown cinchona a sufficient amount of cheap febrifuge to meet India's needs.

Following the extensive development of cultivation in Java and increased demands in India, this ideal of self-sufficiency was abandoned except to the extent that, in 1912, the Government of India decided to build up a reserve of 250,000 lbs. to provide for the contingency of a considerable increase in consumption and to guard against the possibility of an increase in world prices. In pursuance of this decision an agreement was made first with Messrs. Howards, Ltd., in 1919 and subsequently in 1921, with a Dutch combine for the supply mainly of bark. This agreement was terminated in 1928.

As stocks held by the Government of India had fallen far below the figure of 250,000 lbs. owing to military requirements during the War, the purchase of bark from abroad under the above agreement was essential. Partly for this reason, but certainly in connection with the question of adopting a policy of industrial development, the ideal of making not only India but the British Empire self-sufficient by developing India's production of quinine was put in the forefront before the War ended. In order to give effect to this policy, the Government of India in 1917 placed Colonel Gage, the Director of the Botanical Survey of India, on special duty to explore the possibilities. As a result of

his recommendations, plantations were started in Burma; those established in the Tavoy District were destroyed by heavy rains in 1921 and 1922 but, in 1923, the existing plantation in Mergui District was started.

The ideal of Empire self-sufficiency lasted from 1918 to 1923. But in December 1923, after a conference of representatives of the Governments of India, Bengal and Madras assisted by public health experts, it was decided :—

(i) that the Government of India's quinine policy should be directed towards supplying the official and trade requirements of the Indian market only. This was probably due to the financial position of the Central Government, for, at that time, the Incheape Committee had just surveyed the field of Central expenditure and had made drastic recommendations for economy. With regard to quinine, they recommended that the Central Government's stocks should not be allowed to rise above 271,000 lbs., the figure at which they stood at the time.

(ii) That the tripartite arrangements then in force for purposes of production, *viz.*, production by the Governments of India, Bengal and Madras, should not be disturbed, but the Central Government should regulate prices so as to ensure uniformity, as also the programme of cinchona cultivation and that, so long as India was mainly dependent for her supplies on foreign sources, there should be no attempt to break away from world prices.

In April 1928, the stock of quinine held by the Government of India had risen to 361,495 lbs. whilst the total annual official demand had dropped to 60,000 lbs. from the War figure of 80,000 lbs. notwithstanding a fall in the price of quinine from Rs. 30-35 to Rs. 18 per lb.

Another conference of representatives of the Governments of India, Bengal and Madras, assisted by the Public Health Commissioner, was convened to review the situation. This conference advised that there was no practical prospect of the Government of India ever being able to capture the market in India and that the policy should be confined to supplying the official or Government demands for quinine for official institutions. They recommended that 250,000 lbs. be set apart as a permanent reserve against any grave malaria epidemic and that the balance of accumulated stocks be utilised for increasing the general consumption in India, an inducement being offered to the provinces to increase their consumption by the Government of India offering to sell quinine at the rate of Rs. 14 per lb. as against the usual price of Rs. 18. The conference also advised acceptance of the recommendation of the Royal Commission on Agriculture for the centralisation of the Cinchona Departments (paragraph 411 of the Report of the Royal Commission on Agriculture, 1928).

The modified ideal of official self-sufficiency was accepted, but the scheme for increasing sales of quinine to Local Governments received no support from these Governments on the plea of lack of funds. In view of their attitude and as it was estimated that stocks by the end of 1928 would amount to 450,000 lbs. the Government of India decided to consult the Governments of Bengal and Madras before reaching any conclusion on the question of centralisation of the production of cinchona and quinine. The conclusion reached on the recommendations of the Royal Commission on Agriculture for more scientific investigation of cinchona cultivation and manufacture of quinine (paragraph 412) was that the only hope that this investigation will be undertaken lies in the formation of a strong Imperial Department.

The Governments of Bengal and Madras were addressed in August 1929 regarding the centralisation of the production of cinchona and quinine. The Government of Madras were agreeable to the proposal, but the Government of Bengal opposed it urging, *inter alia*, that a definite decision should be postponed until the future constitution of the Central and Provincial Governments had been settled. These objections, added to the financial inability of the Government of India to spend Rs. 61,00,000 on the acquisition of the plantations of the two Local Governments, led the Government of India to decide in September 1931 against any action being taken on the Royal Commission's recommendations for centralisation.

Whilst the Government of India's policy so far had been to make India self-sufficient, by 1930, this objective was limited to one of satisfaction of official requirements. Two factors contributed to this decision :—

- (i) Failure of the official demand to absorb the existing official output. This is obvious from Table I.
- (ii) Absence of any power in the Government of India to compel Local Governments to increase consumption. Without this, the mere power to regulate prices and production, which the Devolution Rules had vested in the Government of India, has availed little to increase consumption.

In order to reduce the financial loss incurred by locking up capital in accumulated stocks, in 1932 another attempt was made to induce Local Governments to buy more quinine by offering it to them at the further reduced price of Rs. 12 per lb. for all quantities purchased over and above their existing average requirements. This offer elicited no better response than that made in 1928, but since then surplus stocks have been reduced chiefly by the free allotment of 45,000 lbs. in 1935-36 to provinces for free distribution.

Financial stringency and failure to increase provincial consumption compelled the Government of India to stop extensions in the Mergui plantations and to retain these only on a care and maintenance basis. Production merely to accumulate stocks could not be justified, especially as the Central Public Health authorities had made the revised recommendation that a reserve of 150,000 lbs. would suffice for any national emergency.

The failure of past efforts to increase provincial consumption by price reduction even to Rs. 12 per lb. suggests that more substantial price reduction is the very essence of the problem. In 1932, the cost of production of Government of India quinine was estimated at Rs. 14.51 per lb. excluding overhead charges for direction and rent, whilst the quinine content of the cinchona bark from Mergui plantations is less than that of the Java bark. It is probable therefore that unless scientific research can improve the quinine content or new plantations elsewhere yield better results, the Government of India cannot hope to produce quinine at a cost which would induce Local Governments to buy more.

The preliminaries to action by the Government of India either to extend their own plantations or to promote research into methods of cinchona cultivation and quinine manufacture would seem to be :—

- (a) to ascertain the true commercial cost of production both in Bengal and Madras; and

- (b) to enquire whether these two Governments will be prepared to treat the production of cinchona and its derivatives as a humanitarian activity rather than a source of profit.

Only when correct information on the first point is available, can it be decided whether the extension of plantations and the promotion of further research are likely to bear fruit. The information so far available indicates that production costs for Bengal are Rs. 10.02 per lb. for quinine and Rs. 9.77 for cinchona febrifuge. The figure given for Madras is Rs. 13-8-10 per lb., but this requires further elucidation before it can be accepted. If these rates on further examination prove to be substantially correct, it is for discussion whether large expenditure on research directed to possible reduction of production costs would be justified. It is to be remembered that the Government of India no longer possess the power to regulate the production price of cinchona and its derivatives.

IV. ESTIMATED NEEDS AND PRESENT CONSUMPTION OF CINCHONA DERIVATIVES IN INDIA.

Although it may safely be stated that in India the present consumption of the cinchona alkaloids is totally inadequate for the needs of the people, it is not possible to do more than to make a rough approximation of the amount required. On several occasions the Public Health Commissioner's reports have stated that the potential demand for quinine lies between 125,000 lbs. and 1,500,000 lbs. per annum.

The number of individuals who suffer from malaria in India every year has been conservatively estimated at a minimum of 100,000,000. For the purposes of mass treatment the amount of quinine should be the minimum effective dose which will relieve symptoms. Taking this at 45 grains, a rough approximation of the annual quinine requirements for India would be $100,000,000 \times 45$ grains or approximately 600,000 lbs.

India's potential demand might also be calculated from the 15 grains *per capita* consumption in Italy and, on this basis, the requirements work out at approximately 700,000 lbs. per annum.

In the 1935 report of the Sub-Committee of the Colonial Advisory Council of Agriculture and Animal Health, it is estimated that 30 per cent of the population in India suffer from malaria and require yearly some twenty-four million ounces of quinine, *i.e.*, 1,250,000 lbs.

From these different approximations it may perhaps be safely assumed that the potential annual demand in India lies somewhere between 600,000 and 1,500,000 lbs. of quinine.

As regards the present consumption of quinine, it is stated in the annual report of the Cinchona Plantations and Factory in Bengal, 1930-31, that 'the total consumption of quinine in India kept remarkably steady at about 211,000 lbs. per annum'. Tables I and II contain figures for (a) production and sales, and (b) imports of quinine salts during the past few years. From these it will be seen that the annual consumption figure has been remarkably steady at about 200,000 lbs. per annum, of which approximately 110,000 lbs. are imported and 90,000 lbs. are produced in India. Present consumption in India is therefore only about one-third of the lower figure for estimated requirements whilst it is less than one-sixth of the higher.

TABLE I.
Production and sales of quinine salts in India.

Year.	GOVERNMENT OF INDIA.		GOVERNMENT OF BENGAL.		GOVERNMENT OF MADRAS.		TOTAL.		Difference between columns 8 and 9.
	Production.	Sales.†	Production.	Sales.	Production.	Sales.	Production.	Sales.	
1	2	3	4	5	6	7	8	9	10
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
1928-29	10,465	12,774	37,077	15,066	18,056	27,832*	65,598	55,672	9,926
1929-30	3,110	12,314	42,776	13,260	22,891	29,009*	68,777	54,583	14,194
1930-31	5,959	19,987	37,678	13,685	20,880	24,355*	64,517	58,027	6,490
1931-32	1,536	16,952	44,029	14,450	19,875	21,563*	65,440	52,965	12,475
1932-33	3,981	11,368	44,052	13,246	23,784	18,290	71,817	42,904	28,913
1933-34	5,740	12,955	45,728	21,252	22,935	20,169	74,403	54,376	20,027
1934-35	3,224	32,284†	52,946	16,088	23,889	23,343	80,077	71,715	8,362
						Total ..	4,90,629	3,90,242	1,00,387

NOTE 1.*—In the years in which Madras sold more quinine than its production, the deficiency was made up by purchases from the Government of India stock.

NOTE 2.†—The Government of India sales, which have been in excess of their production, have, to the extent of the difference, been made up of supplies from the surplus stock.

NOTE 3.‡—Includes 10,000 lbs. sold to Ceylon Government and also sales to Carnegie Bros. and to the trade in India.

NOTE 4.—On an average 65,896 lbs. of bark were harvested annually from the Government of India cinchona plantation at Mergui. This quantity is equivalent to about 2,000 lbs. of quinine.

TABLE II.
Showing imports into India of quinine salts during 1931-32 to 1935-36.

Year.	Quantity.	Value.
	lbs.	Rs.
1931-32 ..	111,056	25,65,369
1932-33 ..	102,660	26,34,493
1933-34 ..	127,572	31,74,199
1934-35 ..	107,628	25,89,966
1935-36 ..	103,610	26,17,842

N.B.—Imports are mainly by trade for private consumption.

V. COSTS OF PRODUCTION AND THE POSSIBILITY OF EFFECTING A REDUCTION IN PRICE.

Before discussing the question of the extension of cinchona cultivation in India, it is necessary to consider the position of the world quinine market. At the present time, total annual world production is said to be about 1,344,000 lbs. The potential supply is, however, much in excess of this figure, as it is understood that Java could readily increase production to 1,400 tons or 3,136,000 lbs. per annum from existing sources. The economics of the situation deserves some notice. Two points appear to be prominent: (1) the extending cultivation of cinchona in Java, although production is meantime being deliberately restricted, and (2) the position of India and the rest of the British Empire which are largely dependent on a foreign source for supplies of quinine.

The present selling price of quinine in India is about Rs. 18 per lb. whilst production costs, according to different reports, show wide variations. The world market is largely controlled by the Kina Bureau which, however, claims that quinine is sold at a price only allowing a 'fair profit' to the producers.

In the Administrative Report of the Madras Government Cinchona Department for the year 1935-36, it is argued that an agreement between the Government of India and the Local Governments is specially necessary in regard to a common issue price throughout India and that the only satisfactory method is to adopt the wholesale price ruling in the market. It was apparently on this understanding that a considerable sum of money was sunk in new plantations in Madras. The report goes on to state that 'there is not the slightest doubt that quinine will never be produced in South India as cheap as in Java. But in these days of economic nationalism it seems but right that India should not abandon the attempt to produce the required quantity of quinine without exhaustive investigation'. In a later paragraph the Madras report states that the factory cost of production during 1935-36 was Rs. 13-8-10 a pound against the unusually low figure of Rs. 11-9-3 in the previous year.

In the 1935-36 report of the Government Cinchona Plantations and Factory in Bengal it is stated that :

'It appears from the trend of discussion in the legislatures and in the press that the public are not so conversant with all aspects of the quinine problem as to make its interest practically effective. Its usual demand has been for more and more and cheaper and cheaper quinine. It knows the remedy and it sees the ravages of malaria, but there are practical difficulties in the way of all attempts to bring quinine at low rates to the great mass of India's sufferers. The present high level of prices in cinchona production is essentially due to the difficulties of production. If the raw material were easy of production, the manufacture of sufficient supplies would be easy enough, but cinchona as a plant is exacting in its demand and it is not everywhere or under any set of conditions that it can be successfully exploited. Costs of production are high, competition is restricted by reason of the climatic and soil requirements of cinchona and these combined explain high world prices'.

The report goes on to say that :

'The public demand for the lowering of quinine prices in India is obviously based on the fact that production costs here are lower than the level of world prices and Governments are therefore urged repeatedly not to make a profit. The difficulty, however, is that the volume of production in India is quite insufficient to meet more than a fraction of the home demand, giving the importer of quinine a virtual monopoly, and as long as this state continues so long will it be impossible for Government to reduce prices below market price without the risk of supplies going astray into the hands of profiteers. So far as the whole of India is concerned, relief can only come from such an extension of local production as would effectively compete with foreign quinine in meeting the total demand and thus maintain prices at a level commensurate with the cost of production at home. It is fortunate

that in India areas exist fairly suitable to the cinchona plant and experience has shown that it can be cultivated here at costs which would allow of a cheapening of quinine for the masses'.

In the same report, production cost of cinchona bark and the cost of extraction, *i.e.*, the total cost per pound of quinine, is given as Rs. 6·548. The market rate for quinine during the year under review was Rs. 22, whilst the Government rate was still Rs. 18 and, at the latter figure, the quinine extracted gave a profit of over Rs. 5,50,000. The figures quoted in the above paragraphs indicate that there is a good margin between cost of production and world prices and that there exists a distinct possibility of producing cheaper supplies.

VI. THE POSSIBILITIES OF EXTENSION OF CINCHONA CULTIVATION IN INDIA.

The Royal Commission on Agriculture remarked that 'if India is to embark on any large campaign of fighting malaria, we are convinced that it will be necessary to reduce considerably the price of quinine within India and this can only be effected if India is self-supporting in production. To achieve this self-sufficiency, a considerable extension of the present area of cinchona will be required'.

Various authorities including the Colonial Advisory Council on Agriculture in London have since had the same question under consideration. The latter body, for example, has suggested the desirability of further experimentation in suitable areas both in East and in West Africa where the cultivation of cinchona has already been shown to be possible. Experience in India has proved that cinchona can be successfully cultivated in selected areas and that India possesses the necessary conditions for production of cinchona to enable her to compete with other producers. In other words, if competition can reduce prices, India is in a strong position to develop that competition. It seems, therefore, that attempts should be made to extend cinchona cultivation in India in order to satisfy the quinine requirements of the country and to avoid the necessity of depending on a foreign country for supplies. In order to ensure success, however, numerous factors must be taken into account. In India, the factor of first importance is suitability of land and climate. The extent and accessibility of areas suitable in these respects, selection of species of cinchona, economy of management and availability and cost of labour are other questions demanding consideration. Organisation for distribution—assuming the cost to Government of distribution is part of the cost of production—also requires attention. Finally, the harvest of bark is not available until the plantation is from 8 to 15 years old. These are all serious deterrents to private enterprise. In view of the previous disastrous failure of private enterprise in Ceylon and South India and of the risks involved, it is certain that Government would have to assume responsibility both for the scientific control and for the expenditure required.

The Bengal Cinchona Department's report for 1935-36 contains relevant remarks on this subject, which may be quoted :

'It is fortunate that in India areas exist fairly suitable to the cinchona plant and experience has shown that it can be cultivated here at costs which would allow of a cheapening of quinine for the masses. When finance is forthcoming, there is no reason why a forward cinchona policy should not be adopted with every prospect of success. Experimental cultivation could be started under suitable conditions in different parts of the country

and all the accumulated experience of the existing cinchona organisations in India would be available to draw upon. But the success of such effort, if it is to be truly national, would seem to depend on a co-ordination of all the provincial efforts. Only certain provinces in India, however, are fortunate in having suitable areas, and with the inauguration of provincial autonomy under the new constitution it would seem that the Central Government alone could bear the responsibility of such a national policy, so that the less fortunate provinces also may benefit. For under the present economic conditions it is not likely nor is it reasonable to ask that those provinces which can produce should make revenue sacrifices in the interest of others. This was probably one of the reasons why the Royal Commission on Agriculture stressed the importance of cinchona as a national problem'.

The report goes on to say :

'The discovery of land and local climatic conditions more suitable to any or all of our species and varieties of cinchona would go some way to a reduction of costs, and such discovery is the first thing necessary towards that development of production that must precede any safe reduction of price. This was recognised when my predecessor, Colonel Gage, toured to find suitable areas. Costs in Burma have been higher than in the Eastern Himalaya and higher than what I believe they would be in the Eastern Himalaya lying between Sikkim and Northern Assam. Experience in the Sikkim Himalaya suggests that if there is to be any centralisation and co-ordination of Indian effort in future, the way towards this is by the examination and experimental proof of a number of selected areas in this Eastern Himalaya sub-alpine tract'.

It may be added here that very recently confirmation of the possibilities of Sikkim has been obtained from an independent source.

In the same report it is also stated :

'The present areas in Sikkim (Mungpoo and Munsong) are clearly ahead of any other tried, and a rational way of going about this business would be to explore the Eastern Himalaya for small unit areas of not less than several hundred acres each at elevations similar to Mungpoo and Munsong, with rainfall also similar and carrying a like natural flora. Unless new areas are found and developed, the future of cinchona production in India is not promising. Mungpoo and Munsong are both old and getting worked out. They will produce for years yet but both are on the decline. From something like 55,000 lbs. of quinine sulphate a year, which we could reach some years ago, we shall be down to something of the order of 40,000 lbs. annually with no prospect of increased production if virgin areas are not immediately taken up. This is the position that has to be faced should it be decided that further exploration, experiment and development should be financed'.

VII. THE POSSIBILITY OF EFFECTING MASS TREATMENT WITH CINCHONA ALKALOIDS IN INDIA.

Experts all over the world have repeatedly stressed the importance of providing cheap and adequate treatment for the malaria sick as the first step in any anti-malarial campaign.

It has nowhere been proved that the eradication of malaria can be effected by the use of drugs alone. Although, however, the provision of treatment is merely a palliative measure, nevertheless it is an effective method of reducing morbidity and mortality and consequently of diminishing the serious economic losses arising from the disease. Experience in many countries has clearly shown that mass quininisation is of great benefit and there can be no doubt that an efficient campaign ensuring adequate treatment to the malaria-ridden people of India would have enormously beneficial results.

As regards the choice of suitable drugs, at the present time the cinchona alkaloids have many advantages, the most important being that they can effect a clinical cure, they can be distributed and used without medical supervision and they are as cheap as, or cheaper than, any other anti-malarial drugs. The question remains as to the form in which cinchona alkaloids can be

employed so as to ensure the greatest benefit to the greatest number for the money available. Three forms are available, viz., quinine, cinchona febrifuge and totaquina.

Quinine and its salts have a high therapeutic value but their cost prohibits their general use. Cinchona febrifuge as originally prepared in India was a mixture of the total alkaloids from the bark of *C. succirubra*. The present febrifuge has approximately the same composition though its method of preparation is somewhat different. The term totaquina is applied to a standardised cinchona febrifuge prepared according to suggestions made by the Malaria Commission of the League of Nations.

In the opinion of many malaria experts, cinchona febrifuge and totaquina are almost as effective as quinine for mass treatment purposes; moreover, they can usually be produced at a lower cost. The possibility that increased demands for cinchona febrifuge would result in a rise in price to approximately that of quinine is a matter which, however, deserves mention. Following a report by the Madras Commission in 1866, that the other alkaloids were as efficacious as quinine in the treatment of malaria, a rapid rise in the price of the former occurred. Apart from changes in relative costs, however, it seems that at present the drug of choice for mass treatment in India is an improved cinchona febrifuge and to satisfy this requirement additional and cheaper supplies of cinchona and its derivatives are urgently necessary.

VIII. MEANS FOR POPULARISATION OF QUININE IN INDIA.

In considering this question it must be remembered that it is necessary not only to educate the people in regard to the value of the drug but to evolve cheap and effective methods for its distribution.

A properly organised selling and advertising campaign extended to the remotest parts of the country should go far to meet both these objectives and adoption of a plan for general quininisation of school children with febrifuge would assist in the same directions.

One of the chief obstacles to effecting a wide dissemination of anti-malarial drugs is in organising a suitable agency for distribution. Existing agencies for distribution include post offices, public health departments and voluntary organisations. As regards the first, the preparation and distribution of the post office packets is an expensive procedure, since the selling price of quinine in this form is approximately Rs. 27 per lb., or almost double the cost of production and manufacture. Presumably the cost of distributing febrifuge through post offices would be equally high, so that this method would largely nullify the benefit expected from the adoption of a cheaper drug. If extension of cinchona cultivation is to be undertaken, during the ten-year period in which additional supplies are being raised, every effort should be directed to the formulation of means whereby cheaper methods of distribution can be effected. In any such scheme, the machinery of distribution of quinine, given free or sold at a price below trade prices, must be so devised and controlled as to prevent profiteering by private concerns or, for that matter, any concern to which the quinine may be supplied at concession rates.

During recent years a great deal has been done by various provincial Public Health Departments in the way of propaganda, with or without free distribution of quinine, and this has been of considerable value. It should be

possible to obtain from Local Governments information as to the methods which were adopted in distributing the supplies of free quinine donated to them in 1935-36 by the Government of India. Information as to difficulties experienced in making satisfactory arrangements and methods found successful would be of value in formulating an effective procedure. Apart from free distribution, the greatest difficulty, of course, has been to place the drug at the disposal of the villager at a price which he can afford to pay. Propaganda meant to induce him to use either post office packets or 'treatments' issued in other ways must largely fail if the drug is beyond his financial resources. The question of price is therefore intimately associated with that of propaganda campaigns and they cannot be considered separately.

It might be possible to do more in the way of distribution of treatments through the agency of voluntary or other lay organisations, either separately or in association with official organisations.

IX. CONCLUSION.

The different sections of this paper will have indicated that the question of the provision of adequate treatment for the malarious sick in India is both wide and complex. It embraces such issues as the advisability of extending cinchona cultivation, the most suitable species to be grown, the selection of areas suitable for their growth, economic repercussions arising from an extension programme, financial considerations, rights under the new constitution, organisation for the distribution of drugs and probably others that have not been mentioned. The question is one in which every province and State in India is intimately and gravely concerned.

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NOTES ON THE DISTRIBUTION, HABITAT, AND FEEDING HABITS OF SOME OF THE FRESHWATER FISHES OF THE BOMBAY KARNATAK.

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I. INTRODUCTION.

A PRELIMINARY note on some of the freshwater fishes found in Dharwar District was communicated by the authors to the 24th session of the Indian Science Congress held at Hyderabad Deccan in January 1937.

Up to the present there are no records in the literature of the freshwater fishes of Dharwar District and the Bombay Karnatak, although the freshwater fishes of neighbouring territories have been studied by Rao and Seshachar (1927). In this paper the freshwater fishes collected in Dharwar and neighbouring districts between April and September 1936 are enumerated.

So far, 29 species have been found in 9 different localities in 3 districts of the Bombay Presidency. It is hoped in a later paper to extend these investigations over a wider area and to systematise these along with the collections which have already been made.

A few of the fishes collected are kept alive in aquaria so that it is possible to study their breeding seasons, feeding habits (including larvivorous habits), and other factors which may be expected to elucidate the ecological influences affecting their growth and distribution.

II. OBSERVATIONS ON FISH COLLECTED FROM TANKS IN DHARWAR DISTRICT.

Dharwar town is the headquarters of Dharwar District. It is situated at an elevation of 2,400 feet above sea level and enjoys an equable climate. The average annual rainfall is 30 inches. There are few rivers in the district, the soil is red, the vegetation rich, and the surrounding country hilly. Tanks are numerous and details of freshwater fishes collected from four of these, lying within a few miles of each other, are given below.

(a) KELGERI TANK.

Kelgeri tank covers an area of about half a square mile and is situated about $1\frac{1}{2}$ miles to the west of Dharwar town. This tank supplies water to the town and is therefore kept more or less free from aquatic vegetation, and fishing is prohibited. In 1936 the tank became almost dry owing to the exceptionally low rainfall. In consequence the fish in the tank were dying in large numbers, and, as this provided a health problem to the municipality, fishing was permitted. Unfortunately, information of these unusual circumstances was not received by the authors before most of the fish had been removed, and it is felt, therefore, that the collection obtained at this time is by no means representative of all the varieties which may normally occur in this tank. From the collections made, fishes belonging to three genera were identified. Details of the genera and species collected are given below :—

- | | |
|----------------------------------|-----------------------------------|
| (1) <i>Barbus chola</i> . | (2) <i>Barbus dorsalis</i> . |
| (3) <i>Barbus melanostigma</i> . | (4) <i>Ophiocephalus gachua</i> . |
| (5) <i>Rasbora daniconius</i> . | |

(1) *Barbus chola*.—This species was collected in April and August 1936, and of the 28 specimens examined, the dimensions of the largest were 98×17 mm. and of the smallest 57×11 mm. The coloration of these fish in the living and dead state is worthy of note. A distinct red spot measuring 2 to 3 mm. in diameter on the posterior end of the operculum appears to be a characteristic feature. In living specimens a faint red medio-lateral band extending from the operculum to the tip of the tail is observed. This red band is much more distinct in the dead fish but in specimens preserved in formalin it disappears within a few days, whereas the red colour is retained when the fish is preserved in a special fixative fluid*.

Eight specimens of this species were kept alive in the laboratory for a time, but all died within six weeks. Each specimen was examined after death and all were found to be heavily infected with trematodes in the gills. It is possible that the high mortality was attributable to the presence of this parasitic infection.

Further observations on this species were made on 9 specimens collected in September 1936 from a tank situated 16 miles to the west of Dharwar town. In these there was great variation in the red coloration of living specimens.

* The authors are indebted to Mr. D. D. Peter Devadas, Fisheries Research Officer, Krusadai Island, for the formula for colour-preserving fluid :—

Gum.....1 oz., Glycerine.....1 oz., Arsenious acid..... $1\frac{1}{2}$ grains, Water.....1 oz.
Specimens are kept for a day or two in the colour-preserving fluid and then transferred to pure glycerine.

In the larger ones the red colour had almost disappeared, the fishes being of a bright silvery white colour. Traces of red were, however, still discernible on the operculum. In young specimens the red medio-lateral bands were conspicuous and the red spot on the operculum was also prominent. The black spot at the base of the caudal fin was very distinct and the dorsal fin rays were black.

It was observed that in the laboratory *Barbus chola* was not so actively larvivorous as *Barbus dorsalis*.

(2) *Barbus dorsalis*.—In April 1936, 21 specimens of *Barbus dorsalis* were collected from the Kelgeri tank and 9 are still alive. Of the preserved specimens the largest measured 61×12 mm. and the smallest 23×5 mm. According to Hora (1936) the coloration of this species differs according to the stage of growth. In very young specimens, not exceeding 23 mm. in length, there are three prominent black spots, one at the base of the dorsal fin, one at the centre of the base of the caudal fin, and a smaller one at the base of the anal fin. These black spots tend to disappear with the growth of the fish, the first to disappear being the anal, leaving the dorsal and caudal spots fairly well marked in specimens measuring up to 83 mm. in length. In larger specimens measuring up to 117 mm. in length, the dorsal spot disappears and the caudal spot is very indistinct. Our observations on the prominence or otherwise of these spots at various stages of growth are given below, and, on the whole, are in close agreement with those of Hora (1936) :—

Dimensions.	Black spot at base of dorsal fin.	Black spot at base of anal fin.	Black spot at base of caudal fin.
23 × 5 mm. ..	small; very distinct.	very small; indistinct.	small; not distinct.
39 × 7 mm. ..	small; distinct.	very small; distinct.	small; distinct.
40 × 8 mm. ..	very distinct.	small; indistinct.	moderately distinct.
42 × 8 mm. ..	distinct.	not discernible.	very indistinct.
42 × 8 mm. ..	indistinct.	not discernible.	indistinct.
46 × 9 mm. ..	not distinct.	not discernible.	not distinct.
50 × 9 mm. ..	moderately distinct.	not discernible.	very indistinct.
50 × 9 mm. ..	moderately distinct.	not discernible.	large; very distinct.
51 × 10 mm. ..	indistinct.	not discernible.	very indistinct.
58 × 10 mm. ..	very indistinct.	not discernible.	very indistinct.
58 × 12 mm. ..	large; distinct.	not discernible.	very indistinct.
61 × 12 mm. ..	small; indistinct.	not discernible.	very indistinct.

With regard to the duration of these spots, especially of those at the base of the dorsal and caudal fins, no alteration in the size or distinctness of these spots was observed in the specimens collected in April after a lapse of eight months. During this eight-month observation period the size of the fish showed

no marked increase. So far it has not been determined whether these fish when reared under natural conditions show any marked increase in size as compared with those reared under laboratory conditions for the same period of time. It may be that the slow growth observed in the laboratory is attributable to the restricted diet of the small crustacea on which they were fed continuously, the only other diet given being mosquito larvæ. The natural diet of this species has not so far been investigated. *Barbus dorsalis* was observed to feed readily on the larvæ of both anopheline and culicine mosquitoes in the laboratory. When mosquito larvæ were introduced into the aquaria it was observed that *B. dorsalis* devoured most of the larvæ introduced even when other species of the genus *Barbus* were also present. No systematic observations have so far been made on the larvivorous habits of this species under natural conditions. It has been observed, however, that in spite of the presence of *Barbus dorsalis* in tanks, mosquito larvæ could be found in large numbers along the edges of the tank especially in small pools or hoof-marks made by cattle. This may be attributable to the fact that the fish were unable to gain access to the larvæ, or that in their natural habitat the fish prefer to feed on material other than mosquito larvæ.

(3) *Barbus melanostigma*.—Only one specimen was obtained.

(4) *Ophiocephalus gachua*.—This is an important edible fish which is found in large numbers in most of the large tanks in Dharwar District. It seems probable that more than one species of this fish is found in this locality but the collections have not so far been subjected to sufficiently detailed study to determine this.

(5) *Rasbora daniconius*.—The largest specimen of this species so far collected measured 111×27 mm. and the smallest 72×13 mm. The smaller species have a distinct thick black line running medio-laterally. In larger specimens this black line is less distinct. This species was found in large numbers in most of the tanks in Dharwar District. *Rasbora daniconius* was observed to be larvivorous under laboratory conditions but no observations have so far been made on their larvivorous and breeding habits under natural conditions.

(b) SOMESHWAR TANK.

In August 1936, specimens of fish were collected from the Someshwar tank near the village of Someshwar. This small tank is much used for washing and drinking purposes and as fishing is not prohibited fish are not plentiful. The tank is covered with thick aquatic vegetation. Representatives of two genera were collected: (1) *Esomus barbatus* and (2) *Rasbora daniconius*.

(1) *Esomus barbatus*.—Only one specimen of this species was encountered, a full description of which will be given in a later communication.

(2) *Rasbora daniconius*.—This species was not so plentiful in the Someshwar tank as in the Kelgeri tank, but the specimens collected were much larger.

(c) NUGIKERI TANK.

The Nugikeri tank is situated about three miles to the south-east of Dharwar town. It is a large tank and aquatic vegetation is abundant along its edges. A temple is situated on the banks of this tank which is used for

bathing purposes by pilgrims, and also for fishing. Four species of fish were collected in April 1936, namely (1) *Barbus chola*, (2) *Barbus ticto*, (3) *Esomus barbatus*, and (4) *Rasbora daniconius*. The two latter species were present in large numbers. *Rasbora daniconius* was observed to be larvivorous whereas *Esomus barbatus* was not. *Barbus ticto* was found to be larvivorous under laboratory conditions.

(d) MUGAD TANK.

The Mugad tank is a large tank situated about four miles to the north-west of Kelgeri tank. Most of the edible fishes available in Dharwar town are caught in this tank. From the few collections which have so far been made the following genera and species have been identified :—

- (1) *Barbus amphibius*.
- (2) *Barbus chola*.
- (3) *Barbus dobsoni*.
- (4) *Barbus ticto*.

Four species of this genus as enumerated were collected, but it is interesting to note that *Barbus dorsalis* which is one of the commonest species found in Dharwar District was not collected from this tank.

(5) *Cirrhina fulunqee*.—This species grows to a fairly large size but its importance as an edible fish has not so far been ascertained. It is not, however, present in large numbers in Mugad tank.

(6) *Callichrous bimaculatus*.—On only one occasion was this species encountered when four specimens were collected. This species grows to a large size and since it has a prominent bulge anteriorly and tapers to the tail it bears a superficial resemblance to the puffy nature of the puffer fish. The food value of this fish has not so far been investigated, nor have the larvivorous habits of its young been studied up to the present time.

(7) *Danio spp.*—On one occasion two specimens of this genus were encountered. Unfortunately they were badly preserved and their specific characters could not be determined.

(8) *Esomus barbatus*.—This species was present in large numbers. Specimens collected in September indicated that at this season the females were filled with eggs and it is probable that this is their breeding season. This species was not observed to devour mosquito larvæ, an observation which is of some interest inasmuch as it has been described as useful for malaria control.

(9) *Lepidocephalichthys thermalis*.—This species was observed to be abundant during the rainy season. It is believed by the local inhabitants to be of value as a cure for rheumatism.

(10) *Ophiocephalus gachua*.—This species is in great demand locally for food. The largest specimen collected was over 2 feet in length. The young are larvivorous but as they grow older they feed on other and smaller fish. The largest specimens are purely carnivorous.

(11) *Rasbora daniconius*.—This species was present in large numbers and was observed to be larvivorous.

III. OBSERVATIONS ON FISH COLLECTED FROM HARIBIDI-HONGAL.

In September 1936, collections of fish were made from a stream at Haribidi-Hongal which is situated 13 miles from Dharwar town on the Soundatti Road. The flow of water in this stream was sluggish and there were large pools in which the movement of water was imperceptible. The bed of the stream was sandy and the edges free of vegetation. Fish were collected with a small drag-net and also with the circular throw-net which is commonly used by the local fishermen. From the collections made the following species were identified :—

- (1) *Aspidoparia morar*.
- (2) *Barbus kolus*.
- (3) *Barilius bendelisis*.
- (4) *Danio neilgherriensis*.

IV. OBSERVATIONS ON FISH COLLECTED FROM KAMBARGANVI.

A special visit was made to Kambarganvi which is situated 16 miles from Dharwar town. Within recent years this locality has become depopulated on account of the exceptionally severe incidence of malaria. Although many small pools and a nullah were examined for fish only one collection was obtained from a nullah supplying water to a garden near the Criminal Tribes Industrial Settlement. The fish obtained were very small and were identified as belonging to the genus *Danio*.

V. OBSERVATIONS ON FISH COLLECTED FROM KOWLGERI.

Kowlgeri is situated 9 miles north-east of Dharwar town on the Soundatti Road. Fish were collected from a small tank in which aquatic vegetation was comparatively abundant, the collections being made with a circular throw-net. The following specimens were identified :—

- (1) *Barbus ticto*.
- (2) *Barilius bendelisis*.
- (3) *Carra mullya*.
- (4) *Nemachilus spp.*
- (5) *Rasbora daniconius*.

VI. OBSERVATIONS ON FISH COLLECTED FROM MAVINKOP.

In September 1936, collections of fish were made from a large tank at Mavinkop which is situated 18 miles from Dharwar town on the Haliyal Road. There is abundant aquatic vegetation along the edges of the tank and fishing is restricted owing to the presence of large numbers of crocodiles. Fish were collected only from the edges and it is possible that the seven different species encountered do not include all the species present. Few specimens of *Barbus* and *Danio* were obtained owing, probably, to the fact that

collection was restricted to shallower waters. The following species were identified :—

- (1) *Barbus amphibius*.
- (2) *Barbus dorsalis*.
- (3) *Barbus sophore*.
- (4) *Barbus ticto*.
- (5) *Danio neilgherriensis*.
- (6) *Danio rerio*.
- (7) *Esomus barbatulus*.

VII. OBSERVATIONS ON FISH COLLECTED FROM MUGADKHAN-HUBLI.

In August 1936, collections of fish were obtained from the Mallaprabha river near Mugadkhan-Hubli village which is situated 30 miles from Dharwar town on the Dharwar-Belgaum Road. Although the rainy season was practically over the water in the river was muddy and the current fairly strong. Fish were collected in large nets operated by two local fishermen. The following species were identified :—

- | | |
|--------------------------------|----------------------------------|
| (1) <i>Ambassis ranga</i> . | (2) <i>Barbus kolus</i> . |
| (3) <i>Barbus jerdoni</i> . | (4) <i>Barbus melanostigma</i> . |
| (5) <i>Barbus ticto</i> . | (6) <i>Chela phulo</i> . |
| (7) <i>Chela boops</i> . | (8) <i>Glossogobius giuris</i> . |
| (9) <i>Mystus cavasius</i> . | (10) <i>Rohitee neilli</i> . |
| (11) <i>Perilampus atpar</i> . | |

The specimens of *Perilampus atpar* were collected from small roadside pools about 150 yards from the banks of the Mallaprabha river. Although at the time when the collections were made there was no connection between these pools and the adjacent fields, it is probable that during the rainy season when the fields were inundated this species had migrated from the fields and were left behind in these pools when the floods subsided.

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The authors are indebted to Dr. S. L. Hora of the Zoological Survey of India, Calcutta, for the loan of literature, for suggesting some of the problems investigated, and for his generous help in the identification of the different specimens encountered; to Dr. B. K. Das, Osmania University, Hyderabad Deccan, for suggesting this field of research; to Dr. B. Sundera Raj, Director of Fisheries, Madras, for the loan of literature and for valuable suggestions; to the Bombay University for financial assistance; and to the Dharwar Municipality for the services of a peon.

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AN ACRIDINE COMPOUND (ACR. X) IN THE TREATMENT OF MONKEY MALARIA.

BY

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[7th October, 1937.]

A PREPARATION which, for convenience in description, we have called Acr. X was sent to us by Sir Upendranath Brahmachari for trial in monkey malaria with the statement that the preparation was 'akin to atebtrin'. No other information, either as to chemical composition or dosage, was given and we proceeded, therefore, entirely empirically, to plan a series of experiments with a view to the comparison of Acr. X with atebtrin for injection (atebtrin musonate)—here called atebtrin for short—from the point of view of their relative efficiency as anti-malarial remedies.

The preparation (Acr. X) was at first supplied to us in tablet form. These canary yellow tablets were readily soluble in water but the solution was not perfectly clear like that of atebtrin. It was considered that this was probably due to some ingredient used in making the tablets and, on enquiry, we were informed that the insoluble ingredient was chalk. This did not interfere with our clinical trials of the drug, since the drug was administered by the oral and intramuscular routes only. However, at our request, we were subsequently supplied with the drug in powder form and from it were able to prepare perfectly clear solutions as in the case of atebtrin.

MATERIALS AND METHODS.

We arranged to test Acr. X and atebtrin under identical conditions in monkey malaria. The strain of monkey malaria used was *Plasmodium knowlesi*, for which we are indebted to Dr. P. F. Russell of the Rockefeller Foundation, International Health Section, and the monkeys used were chiefly *Silenus sinicus* though a few *S. rhesus* were included.

A series of four experiments was carried out and these are considered individually below.

EXPERIMENT I.

Twelve monkeys were divided into two lots of six, one lot to be treated with Acr. X and the other with atebirin.

In each group of six, three monkeys received the drug by intramuscular injection and three by the mouth. As the monkeys were fairly uniform in size, the average weight being about 3 kilogrammes, a flat dose was given. This was fixed at 6 milligrammes per kilo body weight by intramuscular injection and twice this dose by the mouth. In practice a solution of each drug was made in the strength of 0.02 gramme per 1 c.c. of sterile distilled water. Of this solution 1 c.c. for intramuscular injection and 2 c.c. for oral administration, respectively, were used daily. The monkeys, after a preliminary examination for possible natural infections, were all infected with *Plasmodium knowlesi* by intraperitoneal inoculation of citrated blood.

By the fourth day after inoculation all the monkeys showed a moderate to heavy infection and treatment was commenced on the lines indicated above, the course of treatment lasting for three days. The object of this experiment was not to cure the animals but merely to compare the results of treatment by the two drugs when an empirically selected quantity was administered. The results of treatment were gauged by the time required to produce sterilisation of the peripheral circulation and the interval between cessation of treatment and the onset of the first relapse. The details of this experiment are given in Table I.

It will be seen from Table I that in the case of monkeys treated with Acr. X one monkey in each of the series treated intramuscularly and orally was parasite-free as regards the peripheral circulation after two days' treatment while the rest took three days to become so. The average for both series was therefore 2.66 days. In the case of monkeys treated with atebirin the intramuscular series averaged 3 days and the oral series 2.33 days.

The shortest interval between the last day of treatment and the first relapse in the Acr. X treated cases was 10 days, both for the oral and intramuscular series, the averages for the two series in days being 10.66 and 11.3, respectively.

In the atebirin treated cases the shortest interval before relapse was 11 days for the oral and 10 days for the intramuscular series, the averages for the two series in days being 11.66 and 11, respectively.

EXPERIMENT II.

This experiment was designed to determine the dosage of each drug which was required to produce disappearance of parasites from the peripheral blood. The dosage given per day was half that in the first experiment and daily treatment was continued until the peripheral circulation was free from parasites. The details of this experiment are given in Table II.

From Table II it will be seen that the interval required to reach a moderate to heavy infection was slightly longer than in the first series and this was correlated with a somewhat milder infection in the animal used for infecting the experimental monkeys, i.e., the infecting dose of parasites was smaller.

In the series treated with Acr. X orally (four monkeys) two monkeys died due to a heavy infection with rapid blood destruction. In the two remaining animals there was an interval of five and four days, respectively, before parasites disappeared from the peripheral circulation. Among the monkeys given Acr. X intramuscularly two were parasite-free after two injections and one after four. The average number of days of treatment required to make the blood parasite-free was 4.5 for the oral group and 2.66 for the intramuscular group.

The average given for the oral group takes no account of the two monkeys which died.

In the series treated with atebirin orally (three monkeys) one was parasite-free after two days' treatment and the remaining two took three days to become parasite-free. Of the monkeys treated with atebirin intramuscularly (two monkeys) both were parasite-free after three days.

The average number of days of treatment to make the blood parasite-free was 2.66 in the oral group and 3 in the intramuscular group.

This gives the following figures for the total amount of each drug necessary to produce sterilisation of the peripheral blood :—

Acr. X, orally, 27 milligrammes per kilo body weight.

Acr. X, intramuscularly, 8 milligrammes per kilo body weight.

Atebrin, orally, 16 milligrammes per kilo body weight.

Atebrin, intramuscularly, 9 milligrammes per kilo body weight.

As regards relapses Table II brings out the following points :—

In the Acr. X oral series the shortest interval before relapse was 4 days and the longest 7 days, the average being 5.5 days.

In the Acr. X intramuscular series the shortest interval before relapse was 6 days and the longest 10 days, the average being 8.33 days.

In the atebirin oral series the shortest interval before relapse was 7 days and the longest 10 days, the average being 8.66 days.

In the atebirin intramuscular series the shortest interval before relapse was 10 days and the longest 11 days, the average being 10.5 days.

The figures show a slight advantage in favour of atebirin, but the number of animals treated is too small to have any significance.

EXPERIMENT III.

In this experiment twelve monkeys were again used. These were divided into two lots of six which were given, respectively, the two drugs in the dose of 6 milligrammes per kilo body weight daily for five days and no distinction was made in dosage between those receiving the drugs orally and those receiving them intramuscularly. In all cases treatment was for five days.

Treatment was commenced as soon as the blood showed a moderate to heavy infection. The details of this experiment are given in Table III.

Table III shows that in the case of Acr. X oral series, two monkeys were parasite-free after 3 days and one after 4 days, the average for the oral series being 3.33 days.

In the case of Acr. X intramuscular series, one monkey was parasite-free after 2 days, and two after 3 days, the average for the intramuscular series being 2.66 days.

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TABLE
Details of

Number of monkey.	Date of infection.	Drug used and the method of treatment.	Dose and duration of treatment.	DAILY BLOOD					
				2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.
7	25-1-1937	Acr. X oral.	12 mg. per kg. of body weight for three days.	-	+	* ++	++	+	-
8	"	"	"	-	+	* ++++	+++	+	-
9	"	"	"	-	+	* ++	++	-	-
1	"	Acr. X intramuscular.	6 mg. per kg. of body weight for three days.	-	+	* +++	+++	+	-
2	"	"	"	-	+	* +	+	-	-
3	"	"	"	-	+	* +++	++	+	-
10	"	Atebrin oral.	12 mg. per kg. of body weight for three days.	-	+	* ++	++	-	-
11	"	"	"	-	+	* ++	++	-	-
12	"	"	"	-	+	* ++	++	+	-
4	"	Atebrin intramuscular.	6 mg. per kg. of body weight for three days.	+	+	* ++	++	+	-
5	"	"	"	+	+	* ++++	+++	+	-
6	"	"	"	+	+	* ++++	++++	+	-

* Treatment commenced.
+ Mild infection.

++ Medium infection.
++++ Heavy infection.

TABLE
Details of

Number of monkey.	Date of infection.	Drug used and the method of treatment.	Dose and duration of treatment.	DAILY BLOOD					
				2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.
21	4-2-1937	Acr. X oral.	6 mg. per kg. of body weight daily until blood was parasite-free.	-	++++	++++	++++	++++	Died
22	"	"	"	-	++	++++	++++	Died	
23	"	"	"	-	-	-	-	-	++
24	"	"	"	-	+	+	++	+++	++++
13	"	Acr. X intramuscular.	3 mg. per kg. of body weight daily until blood was parasite-free.	-	-	-	-	-	+
14	"	"	"	+	+	+++	++++	+	+
15	"	"	"	+	++	+++	+++	-	-
18	"	Atebrin oral.	6 mg. per kg. of body weight daily until blood was parasite-free.	-	+++	+++	++++	+	-
19	"	"	"	-	-	+	+++	+	-
20	"	"	"	-	-	-	-	-	+
16	"	Atebrin intramuscular.	3 mg. per kg. of body weight daily until blood was parasite-free.	+	++	+++	+++	+	-
17	"	"	"	+	++	++++	++++	+	-

* Days of treatment.

+ Mild infection.

++ Medium infection.

++++ Heavy infection.

II.

Experiment II.

EXAMINATION.									Dosage to make peripheral blood parasite-free.	DAY OF FIRST RELAPSE.†					
8th day.	9th day.	10th day.	11th day.	12th day.	13th day.	14th day.	15th day.	16th day.	17th day.	18th day.	19th day.	20th day.	21st day.	22nd day.	23rd day.
+	+	+	+	+	-	-	-	-	-	30 mg. per kg. of body weight.	+	+	+	+	-
+	+	+	-	-	-	-	-	-	-	24 mg. per kg. of body weight.	+	+	-	+	+
+	+	-	-	-	-	-	-	-	-	6 mg. per kg. of body weight.	-	+	+	-	-
-	-	-	-	-	-	-	-	-	-	12 mg. per kg. of body weight.	+	-	-	+	-
-	-	-	-	-	-	-	-	-	-	6 mg. per kg. of body weight.	-	+	+	-	-
-	-	-	-	-	-	-	-	-	-	18 mg. per kg. of body weight.	+	+	+	+	-
-	-	-	-	-	-	-	-	-	-	18 mg. per kg. of body weight.	-	+	+	-	+
+	-	-	-	-	-	-	-	-	-	12 mg. per kg. of body weight.	++	+	-	-	+
-	-	-	-	-	-	-	-	-	-	9 mg. per kg. of body weight.	-	+	+	-	-
-	-	-	-	-	-	-	-	-	-	9 mg. per kg. of body weight.	-	-	-	+	-

† Relapses were treated with the same dose until parasite-free.

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TABLE
Details of

Number of monkey.	Date of infection.	Drug used and the method of treatment.	Dose and duration of treatment.	DAILY BLOOD					
				2nd day.	3rd day.	4th day.	5th day.	6th day.	7th day.
31	13-3-1937	Acr. X oral.	6 mg. per kg. of body weight daily for 5 days.	-	+	+	* ++++	++++	++
32	"	"	"	-	-	+	* +++	+++	+
33	"	"	"	-	+	++	* +++	+++	+
26	"	Acr. X intramuscular.	"	-	-	-	-	+	* +++
27	"	"	"	-	-	+	* ++	++	+
28	"	"	"	-	-	+	* +++	+++	+
34	"	Atebrin oral.	"	-	+	+	* +	+++	++
35	"	"	"	-	+	+	* +++	+++	+
36	"	"	"	-	-	+	* ++	++++	++
25	"	Atebrin intramuscular.	"	-	-	-	-	-	* +
29	"	"	"	-	-	+	* ++	+	+
30	"	"	"	-	-	+	* +++	+++	+

* Treatment commenced.
+ Mild infection.

++ Medium infection.
++++ Heavy infection.

III.

Experiment III.

EXAMINATION.					DAY OF FIRST RELAPSE.†														
8th day.	9th day.	10th day.	11th day.	12th day.	15th day.	18th day.	19th day.	20th day.	21st day.	23rd day.	24th day.	25th day.	26th day.	27th day.	28th day.	29th day.			
+	-	-	-	-	+	+	-	+	+	+	-	+	+	-	-	.			
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Died of diarrhoea.			
-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	Do.			
+	-	+	-	-	-	-	-	-	+	-	+	-	-	++	+	..			
-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	..			
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	..			
+	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	..			
+	-	-	-	-	-	-	-	-	+	+	++	++	-	-	-	..			
+	-	-	-	-	-	++	+	+	+	-	-	+	-	+	+	..			
+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	..			
-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	Died of diarrhoea.			
-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	..			

† Relapses were treated with the same dose until parasite-free.

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TABLE
Details of

Number of monkey.	Date of infection.	Drug used and the method of treatment.	Dose and duration of treatment.	DAILY BLOOD						
				24/5 4th day.	25/5 5th day.	26/5 6th day.	27/5 7th day.	28/5 8th day.	29/5 9th day.	4/6 15th day.
37	20-5-1937	Acr. X intramuscular.	6 mg. per kg. of body weight daily for 5 days.	* ++	+	+	-	-	-	-
38	"	"	"	* ++	+	-	-	-	-	-
39	"	"	"	* ++	+	-	-	-	-	-
40	"	"	"	* +	+	+	-	-	-	-
41	"	"	"	* +++	+	-	-	-	-	-
42	"	"	"	* +++	+	-	-	-	-	Died of diarrhoea.
43	"	Atebrin intramuscular.	"	* +	+	+	-	-	-	-
44	"	"	"	* +	+	+	-	-	-	-
45	"	"	"	* ++	+	-	-	-	-	-
46	"	"	"	* +++	+	+	-	-	-	-
47	"	"	"	* ++	+	-	-	-	-	-
48	"	"	"	* +	++	+	+	-	-	-

* Treatment commenced.
+ Mild infection.

++ Medium infection.
+++ Heavy infection.

In the atebtrin oral series all the monkeys took 4 days to become parasite-free, the average, therefore, being the same figure.

In the atebtrin intramuscular series, one monkey was parasite-free after 2 days and two after 3 days, the average for the intramuscular series being 2.66 days. As regards relapses it will be seen that in the Acr. X oral series all relapsed, the shortest period before relapse being 10 days and the longest 18 days, the average being 14 days. In the Acr. X intramuscular series one monkey relapsed after 14 days, one after 15 days, and one had no relapse over an observation period of one month.

In the atebtrin oral series all relapsed, the shortest period before relapse being 9 days and the longest 14 days, the average being 11.66 days.

In the atebtrin intramuscular series again all relapsed, the shortest interval before relapse being 14 days and the longest 16 days, the average being 14.66 days.

These figures show a slight advantage in favour of Acr. X, especially as regards preventing or postponing relapses but, again, the number of animals is too small to make the figures significant.

EXPERIMENT IV.

This experiment was designed to determine the comparative cure rate in *P. knowlesi* infection of *S. sinicus* monkeys when equal numbers were treated with the same dose of the two drugs. The cure treatment decided on was intramuscular injection of 6 milligrammes per kilo body weight daily for five days, a rest of one week's duration, and a repetition of the first five days' treatment. Six monkeys were allotted to each drug. The details of this experiment are given in Table IV.

In the Acr. X series four monkeys became parasite-free in 2 days and two in 3 days, the average period being 2.33 days. In the atebtrin series two monkeys became parasite-free in 2 days and four in 3 days, the average period being 2.66 days. One monkey in each group died of diarrhœa during the observation period after the second treatment course.

In the Acr. X series of five remaining monkeys, two relapsed on the 16th and 17th days after cessation of treatment. The remainder have remained parasite-free over a prolonged observation period.

In the atebtrin series of five remaining monkeys two relapsed on the 15th and 17th days after cessation of treatment. The remainder have remained parasite-free over a prolonged observation period. In this experiment, therefore, the behaviour of the two drugs was identical.

Acr. X was examined for us by Mr. H. Hawley, M.Sc., F.I.C., Government Analyst, Madras, and his conclusion, after comparing it with atebtrin*, was that its composition was probably identical with that of the latter drug.

Even although the two drugs may be identical, it was considered worth while to publish our observations because they might be of interest even from the point of view of the action of atebtrin on monkey malaria.

* The comparison is with *atebrin* and not *atebrin for injection*.

SUMMARY AND CONCLUSIONS.

1. The two drugs, Acr. X and atebirin, have been tested in cases of monkey malaria due to *P. knowlesi* under identical conditions.

2. In the production of cures, rate of sterilisation of the peripheral blood, and prevention of relapses they behave in a manner which appears to be identical so far as can be judged by the small numbers of animals experimented with.

3. None of the experimental animals showed any untoward symptoms such as hæmoglobinuria, hæmaturia, or jaundice in the case of either drug.

4. It is our opinion that the preparation supplied us by Sir Upendranath Brahmachari (Acr. X), while not of a composition identical with atebirin for injection, is almost certainly derived from the same base and has an identical action on *Plasmodium knowlesi* infection in monkeys.

ACKNOWLEDGMENT.

We are indebted to the Haverø Trading Co., Ltd., Bombay, which through its representative in Madras kindly supplied us with the atebirin for injection used in the course of our experiments.

ABSTRACT.

NOTES ON THE ANOPHELINE MOSQUITOES OF VILLAGES ON THE ARAKAN COAST OF BURMA*.

BY

R. S. GREWAL, L.M. & S. (S'pore), F.R.F.P.S. (Glas.),
(District Medical Officer, Sandoway).
(MS. 4 pp. with 2 photographs.)

[29th June, 1937.]

As the title indicates this paper records the species of anopheline mosquitoes encountered in villages along the Arakan coast of Burma, and gives information regarding the chief breeding places of each species. This information from a locality in which the anopheline fauna has not previously been adequately studied is summarised below.

Anopheles vagus was the most prevalent species. Larvæ appeared in very large numbers during intermissions in the heavy monsoon rainfall. The larvæ are washed away when heavy rainfall occurs. Following a cyclone, most of the wells in the villages were filled with salt water and the local inhabitants resorted to digging surface wells about 2 feet in diameter and 4 feet in depth in which *A. vagus* was found to breed in profusion. Other breeding places included pools, ditches and drains. The adults of this species were found in large numbers in human habitations.

Anopheles maculatus was less prevalent than *A. vagus*. Its breeding places included pools, wells and drains containing storm water.

Anopheles culicifacies was commonly found breeding in association with *A. maculatus* and also in paddy fields. It is stated that this species was responsible for an epidemic of malaria in Kalayaung village following a cyclone.

Anopheles minimus was rarely encountered in this locality but larvæ were found in paddy fields and in running water in drains.

* Copy of the original manuscript has been placed in the Library of the Malaria Survey of India, Kasauli. This is available on loan to workers who wish to consult the original. (Editor.)

Anopheles kochi was also a rare species in this locality. Larvæ were found in grassy pools and depressions containing water.

Anopheles tessellatus was very rarely encountered. No adults were collected but larvæ were found in temporary pools and swamps.

Anopheles aitkeni was seldom seen. Larvæ were collected from shaded pools, wells and ditches.

Domestic breeding places of various kinds, such as earthenware vessels and old tins, were frequently found to harbour larvæ of *A. vagus* and *A. culicifacies*.

H. W. M.

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